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RECENT DEVELOPMENTS IN THE TRANSPARENT-CHAMBER TECHNIQUE AS ADAPTED TO THE MOUSE [1]

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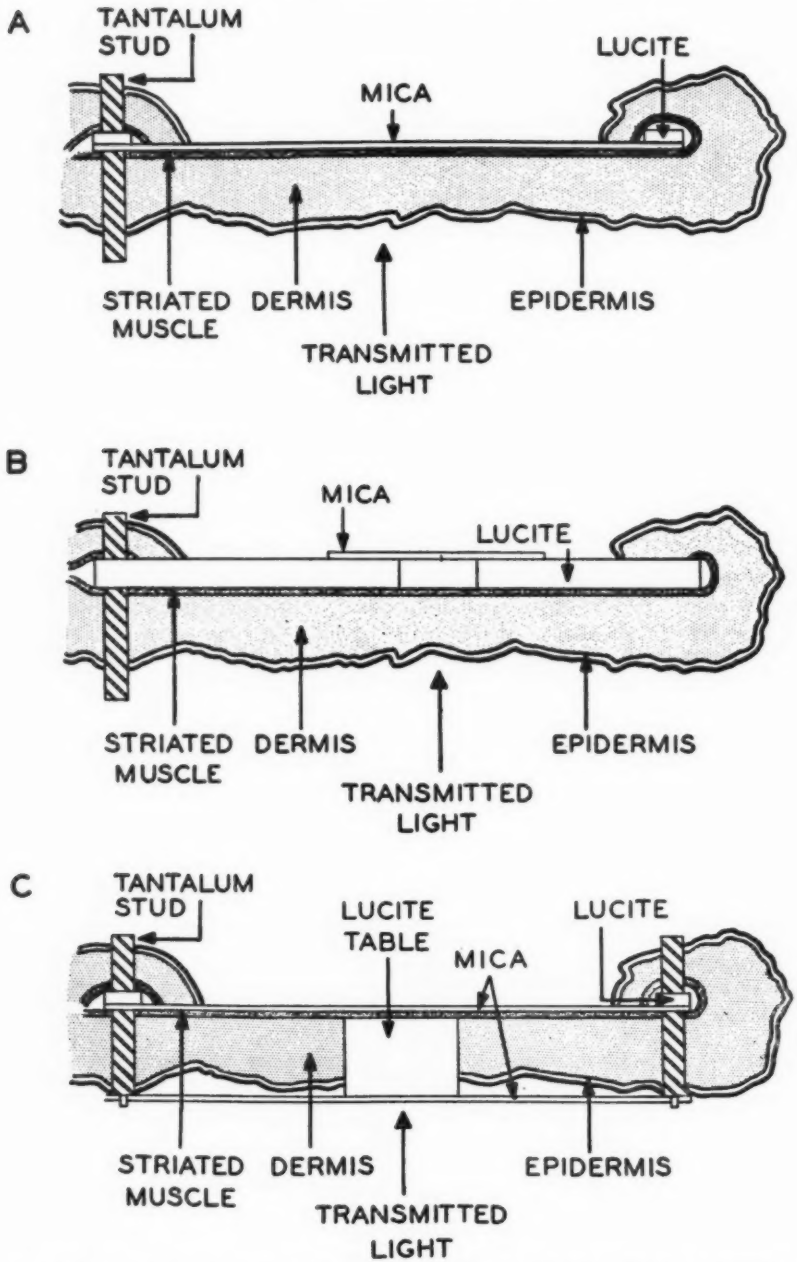
Adaptation to the mouse of the transparent-chamber technique (*1*) has been followed by its application to a variety of problems concerned with the growth, vascular development, and circulatory physiology of normal and neoplastic tissues (*2–5*). This in vivo microscopic approach to problems of tumor induction and growth is directed toward a dynamic, functional study of changes in living tissues. In these studies morphologic observation of the living tissues has been supplemented by measurements of changes in the percentage of functional blood vessels, using the method described by Chalkley (*6*). Methods have been developed (*7*) for measurements of peripheral blood pressure in arteries, veins, and capillaries of normal and neoplastic tissues, and for recording frequency and duration of arteriolar vasomotor activity.

Numerous improvements in the technique have been made in the course of its application. The improvements are concerned with increasing the useful duration of the chambers, modified construction details, shortened operative procedures, increased resolution of cellular detail through improvements in microscopy and through devising chambers in which thin layers of living tissue are made accessible to observation, and improved designs of photographic equipment and procedures for still and motion photomicrography. The present article is concerned with a detailed description of the procedure as now used, based on continued use over a period of 7 years.

CONSTRUCTION OF TRANSPARENT CHAMBER UNITS

Several types of transparent chambers have been designed for specialized purposes. Three of these will be described in this article (Pl. 45, fig. 1, and text-fig. 1) and are designated as the preformed-tissue type, access type, and round-table type.

[1] Received for publication May 3, 1949.



TEXT-FIGURE 1.—Section of skin fold showing relationships of tissues to the chamber. *A*, preformed-tissue type; *B*, access type; and *C*, round-table type.

In all designs the chamber is inserted between the two layers of a dorsal skin fold of the mouse so that a mica cover slip is brought into contact with the innermost surface of one skin layer. The preformed-tissue type (text-fig. 1, *A*) is designed for studies of the histophysiology of tissue growth and reactions. The access type (text-fig. 1, *B*) permits application of chemical agents in direct contact with tissues or allows micromanipulative interference. The round-table type (text-fig. 1, *C*) provides a thin layer of living tissue for studies of cell structure and behavior at high magnifications of the compound microscope.

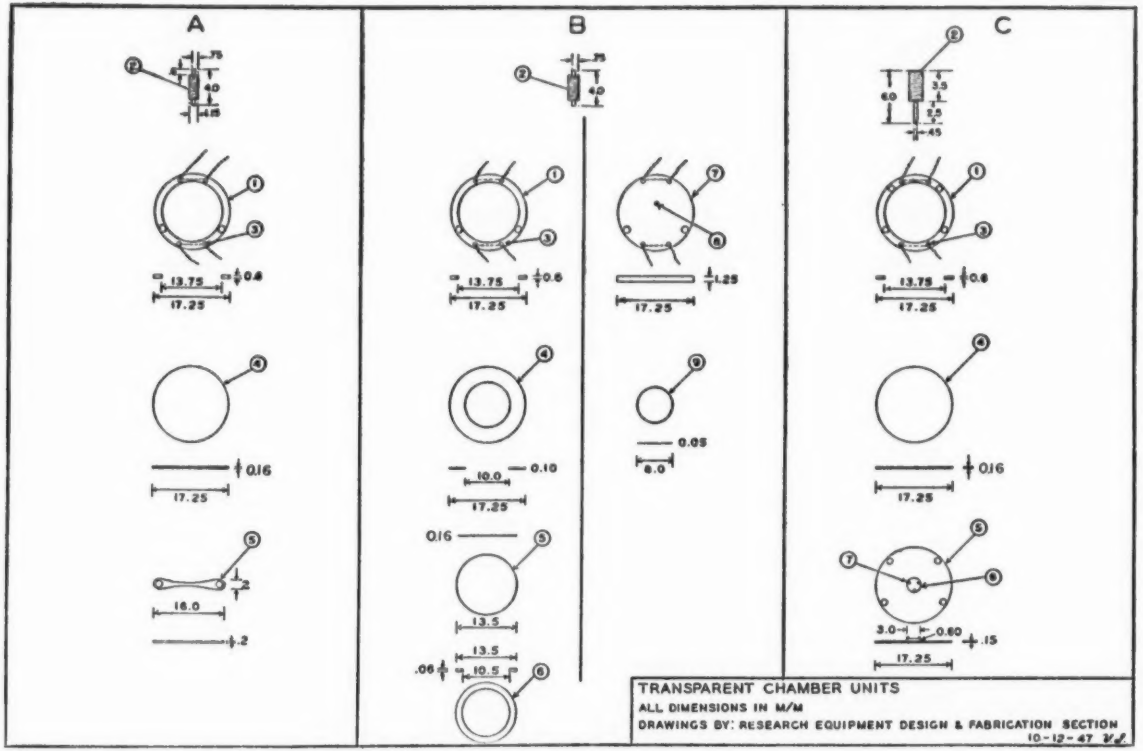
Preformed-Tissue Type

Detailed dimensions of the preformed-tissue type of transparent chamber are indicated in text-figure 2, *A*. Each unit is presented in both the vertical and horizontal planes. The chamber consists of a lucite ring to which is cemented a cover slip of either mica or glass. The details of construction are as follows: Lucite rings (1) are turned on a lathe from lucite rod (¾-inch diameter). Using a brass drill-jig, two large holes are drilled (No. 59 drill) to receive the tantalum studs (2). Smaller holes are made as indicated (3) (No. 77 drill) to receive the two tantalum suture wires.[2] A knife is used to cut a shallow groove connecting the two holes. The wires are then counter-sunk into the grooves. Fine needles [3] are attached to the two free ends of each wire, leaving a wire length of twice the needle length. The two larger holes previously drilled in the lucite ring are threaded to receive the studs (tap wire size .040 inch, 100 threads/inch). Glass or mica [4] disks (4) (0.16–0.18 mm. thickness) are attached to the lucite ring by means of flexible collodion and allowed to dry over night under lead weights. The studs (2) are made from tantalum rod [5] (0.045 inch diameter) and threads are cut along the shaft. The narrower tips of the studs have been found useful but not essential in preventing tissue damage from pressure of the splint. The studs are threaded into the lucite ring and sealed in place with a 5-percent solution of lucite dissolved in chloroform, which is applied also to the rims of the mica and lucite ring. From a sheet of plastic 0.2 mm. in thickness a piece is cut of the shape shown (5) and drilled at each end, as indicated. This piece serves as a support for the skin fold in a manner which will be described later.

Access Type

The access type of transparent chamber (text-fig. 2, *B*) is of two designs. The design shown on the left is similar in construction to the preformed-tissue type chamber except that the mica coverslip (4) contains a central hole for access by means of micromanipulative instruments. The central hole is covered by a mica coverslip (5) which fits into the central hole of

[2] Tantalum wire, 0.003 inch diameter obtained from Ethicon Suture Laboratories, New Brunswick, N. J.
[3] Kirby's vein suturing, very fine straight No. 1350, Kny-Sheerer Corp., Long Island, N. Y.
[4] Obtained from Asheville Mica Co., Newport News, Va., in random thickness and split to desired thickness.
[5] Obtained from Fansteel Metallurgical Co., Chicago, Ill.



TEXT-FIGURE 2.—Construction details of transparent chamber units. *A*, Preformed-tissue type; *B*, Access types; *C*, Round-table type.

lucite ring (1). The mica is held in position by a close-fitting lucite ring (6) which is slipped in on top of it.

The second design of access type chamber consists of a lucite disk (7) (1.0 mm. thick) in which is drilled an access hole (8) for the introduction of chemical agents. This hole is covered by a mica disk (9) sealed on by means of mineral oil. A plastic support (text-fig. 2, *A*, (5)) is also used with this design.

Round-Table Type

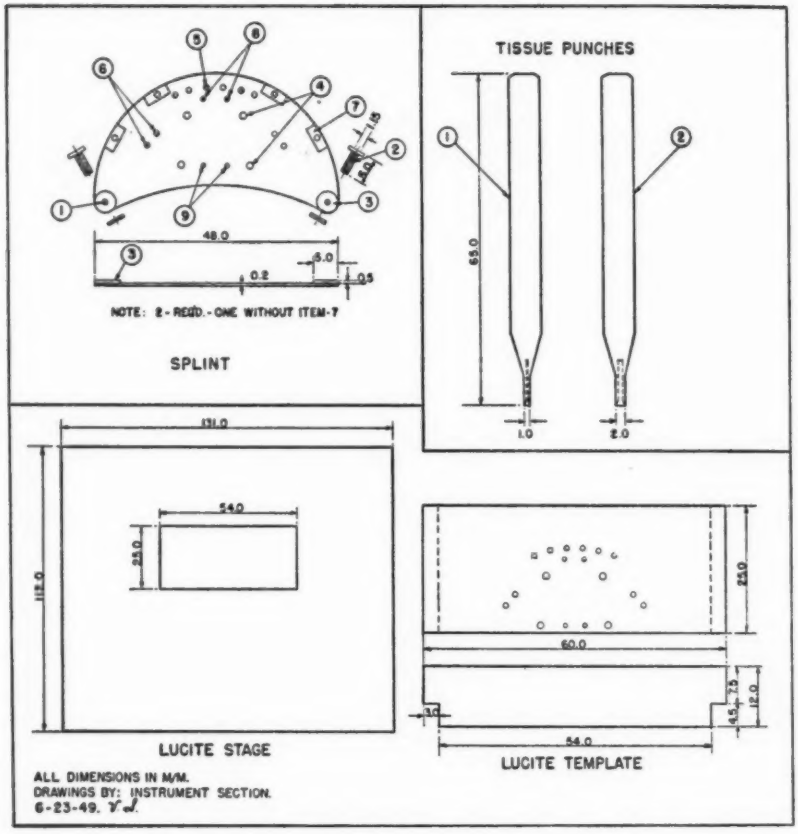
The round-table type (text-figs. 1, *C*, and 2, *C*) is similar to the preformed-tissue design in having a front piece consisting of a lucite ring (1) and mica cover (4). In addition, however, it has a mica rear disk (5) supporting a round lucite table (text-fig. 2, *C*, (6)), the top and bottom surfaces of which are polished. This table is 3 mm. in diameter and 0.6 mm. high. When the chamber is inserted, the lucite table extends up through the tissue so that its top surface lies 40 micra below the inner surface of the top mica disk. This distance is fixed by means of three small lucite spacers attached to the table (text-fig. 2, *C*, (7)). The relative position of the top and bottom of the chamber is adjusted with final accuracy by means of the studs (2). The lucite spacers (7) are attached to the round table by means of chloroform. These are cut from lucite sheet 0.04 mm. in thickness. This thin sheet is obtained by slow evaporation of a thin layer of 1-percent solution of lucite in chloroform.

As this design chamber is used, tissue can be allowed to grow into the shallow space between the top of the lucite table and the inner surface of the mica cover. If desired when the chamber is inserted into the mouse, a thin layer of preformed muscle tissue may be left between the table and the mica cover. If this is done, the spacers are omitted.

Splints

Text-figure 3 shows the plastic [6] splints which were designed not only to protect the skin from traumatic injury but also to give support to the chamber and to maintain it in an upright position. Two splints are used for each mouse, one in front and the other in back of the skin fold. Holes are drilled in these in an identical pattern. Using a brass drill-jig two holes (1) are drilled (No. 56 drill) for the splint bolts (2) and rubber washers (3). Four holes (4) of the same size are drilled in the central portion of the splint, the lower two for passage of the chamber studs and the upper two to serve as guides in the operation. These holes are matched with the large holes in the lucite rings (text-fig. 2, (1)). A No. 78 drill is used to make the remaining holes, six near the periphery (5), two at each side (6), and two each at top (8) and bottom (9), which allow for passage of suture wires during the operations. The rubber washers (3) are punched from sheet rubber, 0.6 mm. thick. These are sealed to the splints using a solution of lucite in chloroform. Tantalum

[6] Plastic cover slip (24 x 50 mm. No. 2 thickness). General Biological Supply Co., Chicago, Ill.



TEXT-FIGURE 3.—Splints, template, and punches.

bolts are then passed through the splint selected for the back. Four spacers (7) made of lucite (2×3×3 mm. thick) are attached by lucite in chloroform to the splint selected for the front, and holes are drilled through these. The spacers later serve to prevent pressure necrosis of the skin.

LUCITE TEMPLATE

During the operative procedures, a rectangular lucite block (text-fig. 3) serves as a medium for transmitting light through the skin fold. It also serves as an anvil against which the holes are punched through the skin to accommodate the bolts and studs. Preformed holes in this lucite block, corresponding to those in the splints, also serve as a template for location of the tantalum sutures and as a guide in alignment of the blood vessels. In use, this template is slipped into the cavity of a special lucite stage (text-fig. 3) substituted for the glass stage plate of a binocular dissecting microscope.

Sterilization

The mica surfaces are cleaned by swabbing with 80-percent alcohol. The chamber units are sterilized in groups of eight. They are placed in a Buchner funnel (6-inch diameter) and are covered with a 1:500 aqueous solution of metaphen [7] for 1 hour. This solution is drained from the funnel and is followed by three changes of sterile distilled water at 1-hour intervals. The units are dried between layers of sterile gauze before being placed in sterile containers.

OPERATIVE PROCEDURE

Preparation of Skin Fold

The operation is carried out under nembutal [8] anesthesia, with aseptic precautions.

The hair of the dorsal skin area is first sheared with the electric clippers then closely shaved with an electric razor. The skin is cleaned with dilute tincture of iodine containing 1 gm. of iodine and 0.8 gm. of potassium iodide per 100 cc. of 80-percent alcohol; it is then rinsed with 80-percent alcohol.

The back splint is aligned with the template and fastened down with Scotch tape. The mouse is placed on its right side against the template on the stage of the dissecting microscope with its head to the left with respect to the operator (Pl. 46, fig. 2).

It is extremely important at this stage of the operation that the larger vessels from the axillary and inguinal regions are arranged so as to enter the site selected for the chamber half way between the center and top of the skin fold (Pl. 46, fig. 3), coming up between the splint bolt and the future position of the chamber bolt. The skin fold is fixed in position by needles, threaded with tantalum sutures .003 inch in diameter, passed through the skin layers and into the back splint and the template. The front splint is passed down over the threaded needles (fig. 2). The entire assembly is lifted from the template and the sutures tied off. Two holes are made with a tissue punch (text-fig. 3) for passage of the splint bolts. Further support is provided by interrupted sutures through the splints and skin fold, using the holes indicated in text-figure 3 (6). The front and back splints are fastened together by passing sutures through holes in the spacers (text-fig. 3 (7)). Completion of this phase of the operation provides a stretched double layer of skin of from 20 to 25 mm. diameter for the insertion of the chamber.

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[7] Obtained from Abbott Laboratories, North Chicago, Ill.

[8] Nembutal powder is weighed out into stoppered vials in 20 mg. quantities. At the time of use, 10 cc. of isotonic saline is added to the vial, making a concentration of 2 mg. per cubic centimeter. Dose varies with weight and strain, but for strain C3H mice weighing 30 gm., it is 0.08 mg. per gram of body weight, or 1.2 cc. of the above solution intraperitoneally. No sterilization of the solution has proven necessary. Any unused solution is discarded.

Insertion of the Transparent Chamber

The mouse is arranged so that the head is to the right of the operator. Using the two lower holes in the central area of the splint as guides (Pl. 46, fig. 3), two holes are punched which penetrate both skin layers. The splints are trimmed as indicated in the dotted lines in text-figure 3, and the mouse is then placed with its head to the left. Four radial incisions are made through the top skin layer, starting at the center of the future chamber site and extending for a length of approximately 5 mm. toward the four holes in the central portion of the splint. The four skin flaps thus formed are reflected by means of small artery clamps (Pl. 47, fig. 4). Using the dissecting microscope, the superficial loose connective tissue is then carefully dissected away from the internal muscular layer (panniculus carnosus) of the underlying skin.

A tissue implant may be made at this time. Tissue from the donor animal is placed on a depression slide within a petri dish containing isotonic saline to delay drying of the tissue. Glass microhooks are used to form a pocket of connective tissue to receive the tissue fragment (0.5 to 1 sq. mm.) teased from the main clump in the petri dish.

The chamber is inserted between the skin layers as the cut skin segments are drawn up over the outer rim. The tantalum studs are slipped into the holes punched in the skin, and any loose connective tissue that may adhere to them is removed. The internal surface of the back skin layer is thus brought into direct contact with the under side of the cover slip. The built-in sutures of the chamber are passed through the front skin layers, tied, put through the front plastic splint, and tied again loosely both top and bottom. The skin edges are then trimmed and the transudate permitted to clot for attachment of the skin to the edge of the chamber. The specially shaped plastic strip (text-fig. 2, *A*, (5)) is placed between the back skin surface and the splint to prevent detachment of the tissue from the mica. The end result is illustrated in figure 1.

The round-table type of transparent chamber (Pl. 47, fig. 5) is designed to provide a thin layer of tissue, rather than the full skin thickness obtained in the preformed tissue type. The operative procedures are similar to those described above for the preformed-tissue type with the following modifications. When it is desired to save a layer of preformed muscle on top of the chamber table, a layer of tissue 2 mm. in diameter is dissected away from the back skin layer so as to leave the thin layer of striated muscle (panniculus carnosus) intact for observation. A lucite table disk (3 mm. diameter) is inserted into this space, and penicillin ointment is applied to the surface of the skin. A mica plate (text-fig. 2, *C*, (5)) is then slipped over the studs, its inner surface brought into contact with the back surface of the table and held in position by the splint.

When no preformed tissue is to be left on the table, but when tissue is allowed to grow in from the sides, a punch is used to cut out a circular disk of skin (2 mm. diameter) before introduction of the chamber. The lucite disk (text-fig. 2, *C*, (6)) provided with spacers (text-fig. 2, *C* (7))

(approximately 40 micra thick) is introduced into this hole. The operation is then completed as described above.

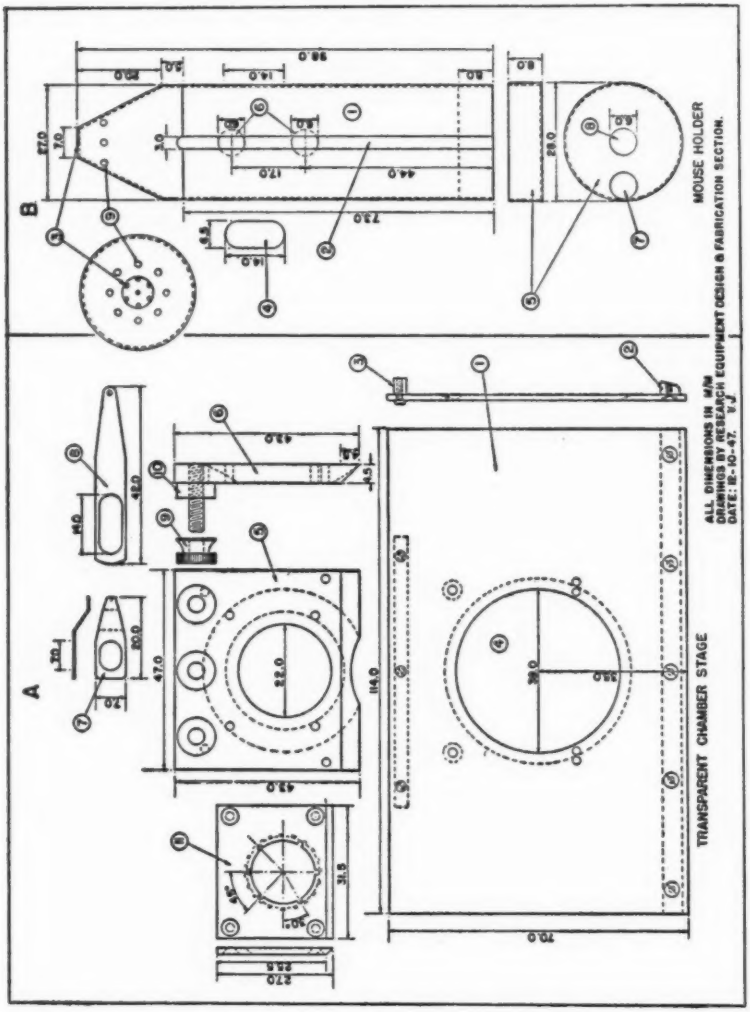
MICROSCOPIC EXAMINATION AND PHOTOMICROGRAPHY

All microscopic studies are carried out on the unanesthetized mouse. A brass or lucite holder constructed as shown in text-figure 4 and plate 48, figure 6 is used to immobilize the animal. The holder consists of a tube with a conical end and with a slot (text-fig. 4 (2)) to permit the chamber and splint supports to remain outside. The holder is closed by a metal cap (5) in which a hole (7) is provided for the tail. The mouse is then placed on a special stage, which is supported on the microscope mechanical stage. The special stage consists of a brass plate (1) with a guide (2) which fits into the beveled slide of the mechanical stage and is made level by means of the spacer (3). A hole (4) in the base plate serves for the passage of light to the chamber. The transparent chamber is supported at the desired level on the stage by means of the support (5) and (6) on top of which is attached the adapter (11), designed to support the projecting chamber bolts in its slotted openings. The chamber is maintained in a horizontal position by means of the spring clips (7) and (8).

Plate 48, figure 7, shows a general view of the examining unit and photomicrographic camera used in the transparent chamber studies. A table with a cast-iron base supports the stereoscopic and compound microscopes. Any one of three light sources can be brought into optical alignment by moving them on a sliding support. In photomicrography the camera bellows (1) is slid forward so as to center an adapter over the optical center of the microscope. A first surface mirror (2) reflects the light at an angle of 45 degrees so as to direct it horizontally to the ground glass (3) at the rear of the bellows. Focusing and selection of field is accomplished by means of remote controls (4) carried from the microscope to the rear of the camera.

A tungsten ribbon filament lamp (5) is used for visual observation at all magnifications. Photographic records are made with a tungsten arc lamp (6). A photo-electric exposure meter, arranged as described by Earle (*8*), is used to determine correct exposure time at the plane of the image. Sodium nitrite solution (0.7-percent aqueous solution in a water cell of 32 mm. thickness) and glass heat filters [9] are used in the path of light in order to prevent damage to the tissues from ultraviolet or infrared radiation. The bottom lens of an aplanatic condenser (0.30 n. a.) is used in low-power observation with the stereoscopic microscope and for magnifications up to 200 × with the compound microscope. For higher magnifications and with oil immersion objectives in examination of the thin layers of living tissues of the round-table type chamber, a two lens Abbe condenser (0.70 n. a.) [10] is used. As an alternative to the 0.70 n. a. condenser, it has been found feasible to use an objective as a condenser. An objective which matches the one used above the preparation is necessary. A special fluorite oil immersion objective [10] (4.3 mm. e. f., 1.0 n. a.

[9] Kopp K–48 obtained from Kopp Glass Co., Swissvale, Pa.
[10] Obtainable on special order from Bausch and Lomb Optical Co.



TEXT-FIGURE 4.—Transparent chamber stage; mouse holder.

and a free working distance of 0.55 mm.) has been found extremely useful.

Photomicrographs are made on Eastman Contrast Process Panchromatic film, size 8×10 inches, and examined as negatives. A Wratten X–1 green filter is used to increase contrast of the living tissue. Development is carried out in DK–76 containing 10 gm. kodalk per liter, for a duration of 8 minutes at 68° F.

A cinephotomicroscope has been completed recently for use with the transparent-chamber technique. Details of this unit will be given in a subsequent article.

Reference to figure 1 and text-figure 1 shows that the coverslip of the chamber is in contact with the inner surface of the back layer of skin; the relationships of the tissues to the chamber are shown in these illustrations. At low magnifications, as in plate 49, figure 8, several tissue layers are seen. In this photograph of a sarcoma within the chamber, the tumor mass is delimited from the surrounding normal tissue by its rich, irregular capillary arrangement and by its translucent appearance. Adjacent to the tumor may be seen branches of the main artery and veins of the subcutaneous layer. The capillaries of the muscle layer are apparent as dark parallel lines, noted especially clearly at the lower left of figure 8.

It should be emphasized that in the preformed-tissue type chamber, microscopic observations using transmitted light are carried out with the light from the condenser coming through one complete skin layer of living tissue approximately 500 micra in thickness. With such a preparation one cannot expect to obtain detail and resolution equivalent to the best in vitro conditions where the thickness of the cell layer is of the order of 1 to 20 micra.

In such preformed-tissue chambers at magnifications up to 50 ×, fat cells, hair follicles, nerve bundles, arteries, capillaries, veins, and lymphatics are easily and accurately recognizable. At magnifications ranging from 200 to 500 × one can focus upon the layer of cells attached to the mica cover, identifying cytoplasmic granules and nuclei of leucocytes, fibroblasts, and macrophages. At deeper levels in the tissue, by selecting satisfactory areas, erythrocytes, leucocytes, and platelets in the circulating blood, endothelial cells lining capillaries, and cross-striations in voluntary muscle can be distinguished. In such structures as smooth muscle the cells cannot be distinguished, although the tissue can be recognized by its contractility. Differences in refractility at cell borders make it possible to distinguish the characteristic architecture of sarcomas. Glandular acini are quite readily identified in transplants of mammary adenocarcinoma, thyroid, and gastric tissue.

Plate 50, figure 9, illustrates an access type chamber in which one can see methylcholanthrene crystals lying on the surface of the muscle layer after having been inserted through the access hole.

The round-table type chamber provides a thin layer of living tissue which can be examined under oil immersion at high magnifications, and with a high degree of resolution of cellular detail. Up to the present time, this design has been used to only a limited degree, and its full potentialities have not been realized. Plate 51, figure 10, shows a group of migrating cells which have wandered into the space over the table. As may be seen, the definition in such a preparation allows study of considerable cell detail. Note, for instance, that what appears to be the periphery of an undulating membrane around these cells can be clearly seen.

Plate 52, figure 11, shows regenerated striated muscle as seen in the round-table type of transparent chamber.

The useful duration of a chamber preparation is approximately 30 days. While the procedures utilized in this laboratory are aimed toward the study of fairly large numbers of mice, simpler procedures of operation and examination can be readily devised. In this laboratory, 2 workers can maintain approximately 12 to 18 mice under daily observation, with measurements and necessary photographic records. Construction of each transparent chamber unit including splints requires about 1 hour, and the operation lasts 1 hour. In skilled hands, failures average less than 5 percent in the routine procedures of the preformed-tissue type chamber. Failures usually are due to infection or to improper alignment of vessels, leading to circulatory obstruction.

SUMMARY

Recent improvements in the techniques for in vivo microscopic study of tissue growth and vascular reaction in transparent chambers in mice are described. These chambers are inserted into a skin fold of the mouse and so constructed that micro implants of tumor cells or other tissues can be made either at the time of operation or subsequently. Three types of transparent chambers have been designed. These are the preformed-tissue type, the access type and the round-table design. Details for construction and insertion of the chamber are given. By the use of this technique it is possible to carry on prolonged microscopic observations of the living tissue (30 to 60 days) at magnifications up to 1,200 ×. Methods have been devised for obtaining photomicrographic and cine-photomicrographic records of the growth and reactions of blood vessels, tissues, and cells in living mice.

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PLATES

PLATE 45

FIGURE 1.—Mouse with preformed type transparent chamber in dorsal skin fold.

1

PLATE 46

FIGURE 2.—Rectangular lucite template serving as a guide for the passage of sutures through splints and skin fold. The back splint is in position.

FIGURE 3.—Relationship of vessels in skin fold to position of chamber, showing 4 guide holes in splint corresponding to location of holes in lucite ring. *A*, Splint bolt. *B*, Hole punched for chamber bolt.

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B
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PLATE 47

FIGURE 4.—Upper skin layer reflected to expose tissue for insertion of implant, which is followed by introduction of chamber.

FIGURE 5.—Surface view showing tissue within round-table type of chamber.

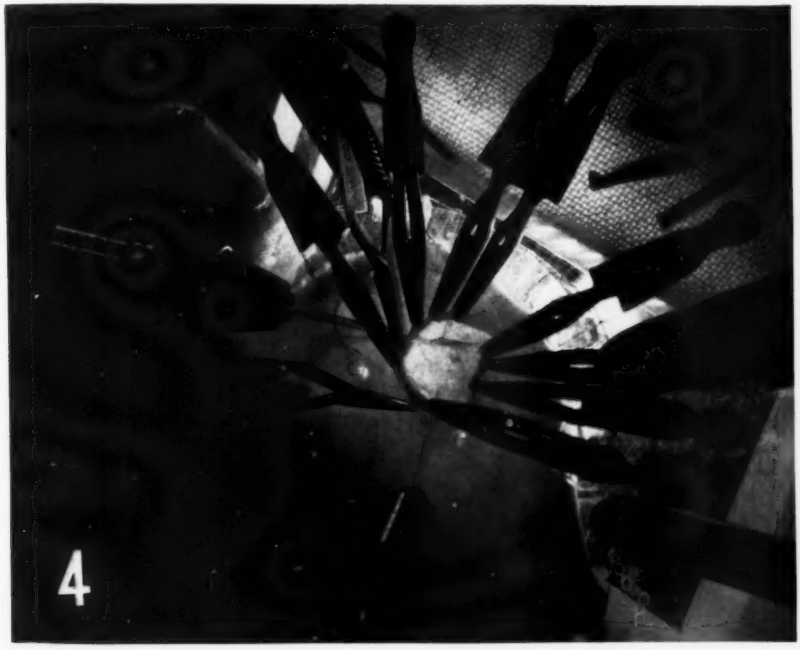
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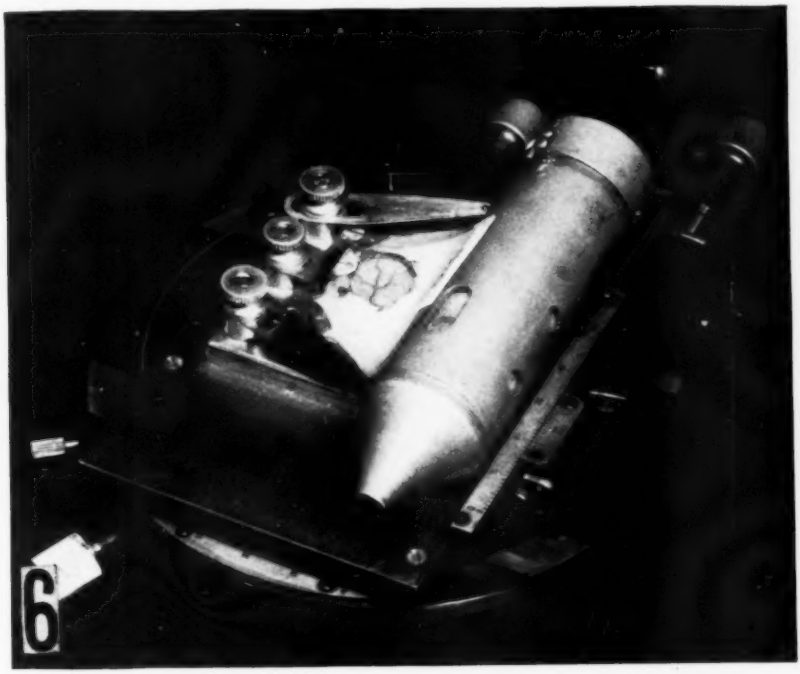
PLATE 48

FIGURE 6. Mouse within holder in position on microscope stage.

FIGURE 7.—Examining unit and photomicrographic camera used in transparent chamber studies.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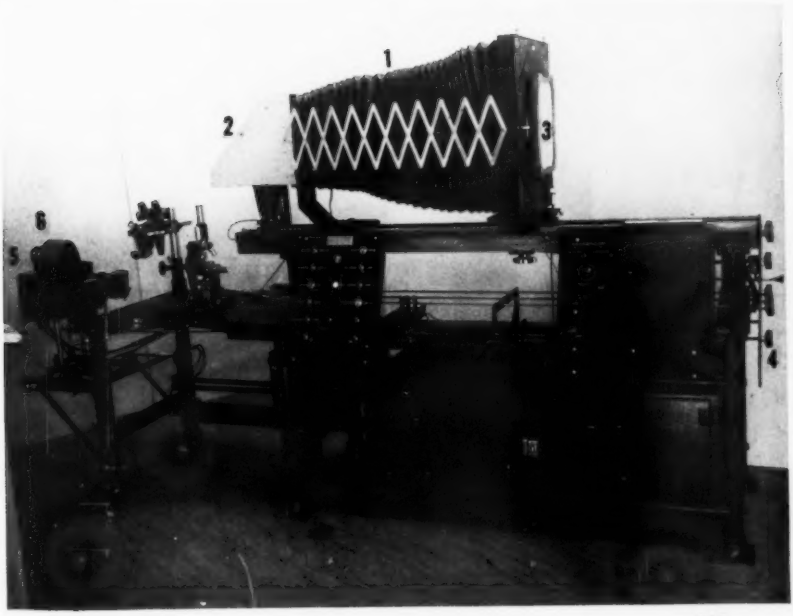
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PLATE 49

FIGURE 8.—Sarcoma surrounded by normal subcutaneous tissue and striated muscle. × 50.

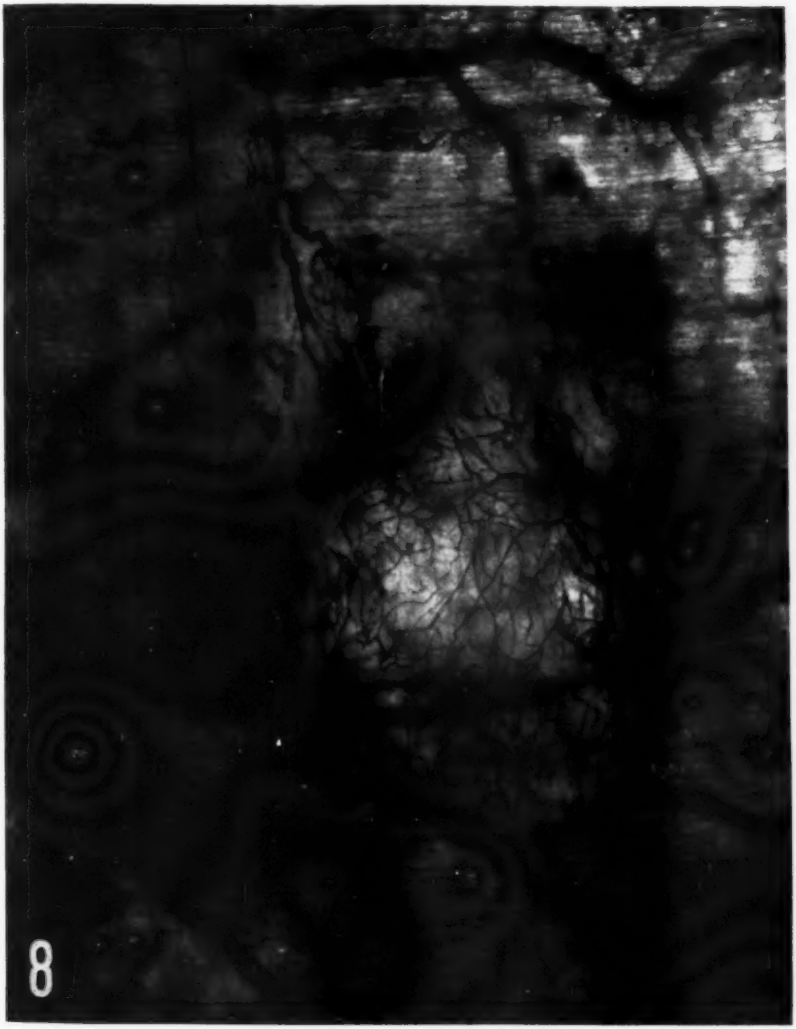
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PLATE 50

FIGURE 9.—Access type chamber. × 50.

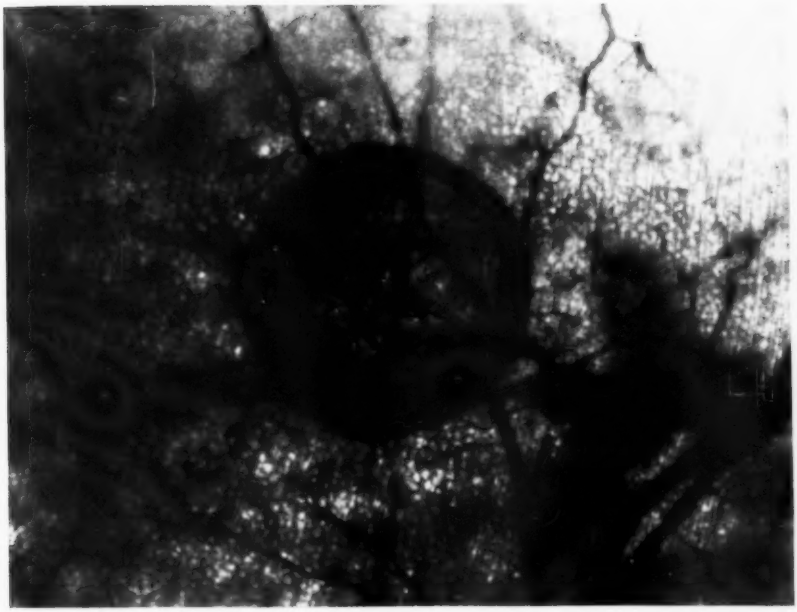

PLATE 51

FIGURE 10.—Cell detail in round-table type chamber. × 1,200. The arrow indicates what appears to be an undulating membrane around these cells.

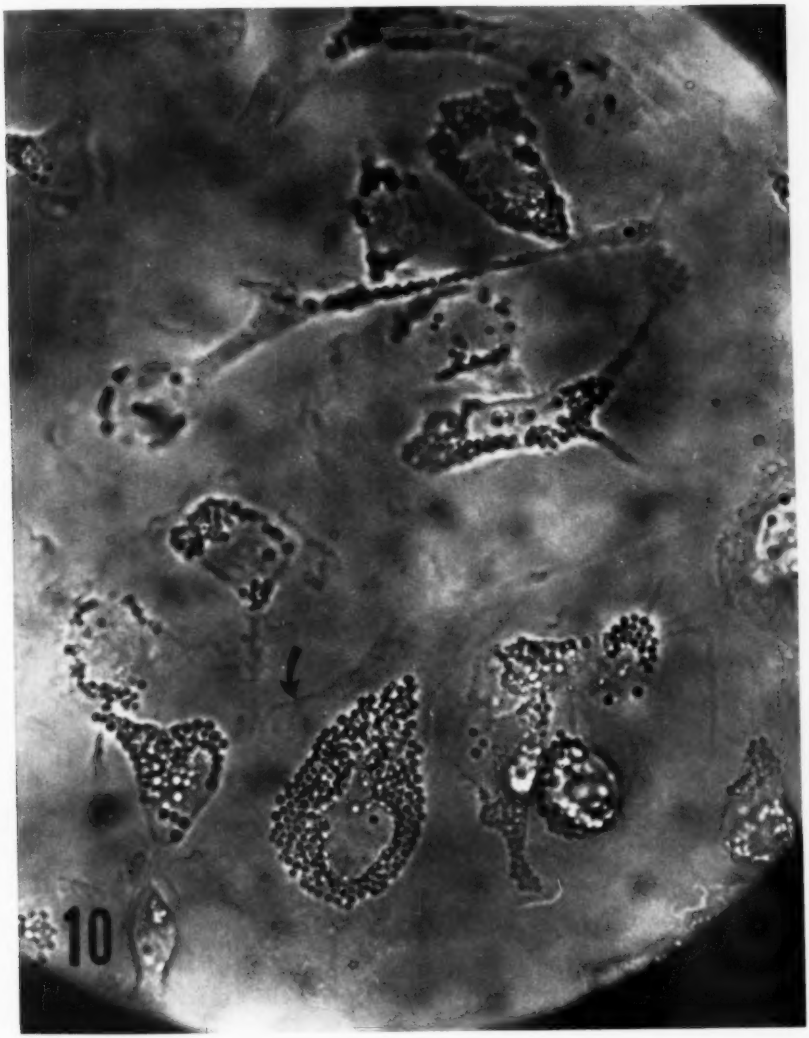
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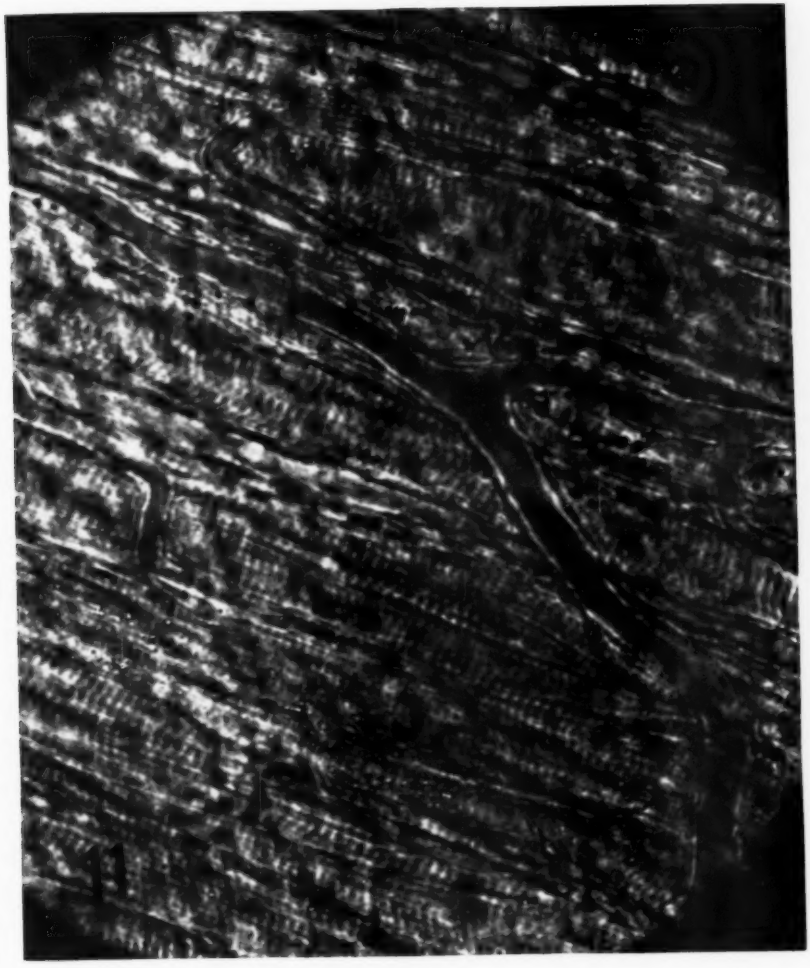
PLATE 52

FIGURE 11.—Cross-striations in regenerated muscle within round-table type chamber. × 1,000.



DEVELOPMENT OF ADENOCARCINOMA OF THE BREAST IN THE WISTAR RAT FOLLOWING THE GASTRIC INSTILLATION OF METHYLCHOLANTHRENE [1] [2]

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The production of carcinoma of the glandular portion of the stomach in a laboratory animal remains one of the most baffling as well as one of the most important problems in experimental cancer. Although a number of investigators have failed in their efforts to produce gastric glandular carcinoma in animals by feeding different carcinogens, we were prompted to make still another attempt because of the need for the production of experimental gastric cancer by a method that might resemble a mechanism that could conceivably be responsible for the spontaneous lesion in man. It has been suggested (*1*, *2*) that the unbroken mucus layer covering the stomach mucosa is probably an important barrier to the action of carcinogens. We thought, therefore, that theoretically suitable experimental conditions for the production of the desired gastric lesion could be achieved (1) by producing and maintaining a break in the protective gastric mucus layer, and (2) by the repeated gastric instillation of a carcinogen. A fundamentally similar approach was tried by Ivy and Cooke (*3*) in rabbits.

Since the distal portion of the stomach is the usual site of cancer of this organ in man, we elected to use the calcium-free diet which Zucker and Berg (*4*) have shown to produce ulceration of the antrum of the stomach when fed to young rats. In addition to producing a break in the gastric mucus layer, in the healing process such lesions would provide young cells to be exposed to the carcinogen (methylcholanthrene) introduced into the stomach. While our efforts, like those of others, failed to produce gastric glandular cancer, we did, nevertheless, see the development of a very high incidence of breast cancer in our female animals.

MATERIALS AND METHOD

Wistar strain albino rats were used. Our colony is derived from a group of pedigreed Wistar rats obtained originally from Wistar Institute. Inbreeding has been carefully avoided at all times. We felt that a successful result in this strain would be especially significant since the incidence of spontaneous malignant tumors is extremely low in the Wistar rat during the age periods covered by our studies. When the experiments were

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started, our rats were between 5 and 7 weeks old and weighed from 60 to 70 gm. Each animal was housed singly in a cage with a bottom of wide mesh; this may have been a fortuitous measure since Andervont (*5*) has shown that crowding will decrease the incidence of mammary tumors in female C3H mice.

Healthy young rats placed on the calcium-free diet deteriorated so rapidly that we were forced to feed this diet intermittently until the animals were approximately 6 months old. The following schedule was employed: Calcium-free diet, 4 weeks; Rockland rat diet, 2 weeks; calcium-free diet, 4 weeks; Rockland diet, 1 week; calcium-free diet, 8 weeks; Rockland diet, 1 week. Thereafter the animals could be maintained uninterruptedly on the calcium-free diet (*6*) which consisted of the following:

Labco casein	27. 70 percent
Cerelose	64. 99
Mod. Wesson salts (free of calcium and phosphorous)	1. 20
KH_2PO_4	0. 11
Yeast extract type II (Standard Brands)	5. 00
Concentrated carotene in oil (7,500 units of vitamin A activity per gram—SMA Division of Wyeth)	1. 00
	100. 00

Methylcholanthrene (minimum melting point 180° corrected) was the carcinogen employed. Two milligrams dissolved in 0.5 cc. of olive oil were instilled into the stomach daily for 6 days a week through a stomach tube using a technique previously described (*7*). Four groups of rats were studied for this report: intact and castrated males and females.

RESULTS

While no grossly visible gastrointestinal tumors were seen in any of our animals, a high incidence of subcutaneous tumors was observed especially in the females. These tumors were grossly of two types. One, which we have designated type A, appeared as a tiny nodule in an area where breast tissue in the rat could be expected normally to be present. If the animal was held suspended by the front legs, these tumors could be observed in silhouette even when they were quite small. The type A lesion grew rapidly and in 3 to 4 weeks after its appearance measured 2 to 3 cm. in diameter. Frequently the tumor grew to such a large size in so short a time that central necrosis of the tumor and ulceration of the overlying skin developed.

The second type, designated type B, was found by palpation only. This lesion when first detected left us uncertain as to the existence of an actual tumor, a doubt which was occasioned by both the character of the lesion and its location. If the normal posterior axillary or anterior groin fold is palpated between the thumb and index fingers, and if this palpation is begun by including the muscles of the upper portion of the limb, the muscle edge snaps away from between the palpating fingers as the

examining fingers are slipped toward the edge of the skin fold, and only the two skin layers are interposed until the edge of the fold is reached. A type B tumor is detected by palpation as a small discoid mass just proximal to the muscle edge between the two layers of the skin in either the posterior axillary or anterior groin fold. This type of tumor grows very slowly and at no time could we detect such a lesion by inspection alone.

The initial group (Group 1) consisting of 13 females and 5 males was started on the calcium-free diet on May 7, 1947. Gastric instillation of methylcholanthrene was begun 1 week later and continued as described above until each animal died or was sacrificed.

The first superficial tumor (type A) was detected in a female rat 181 days after methylcholanthrene was started, and the latest appeared at 418 days in another female. Single tumors appeared in 2 of the females; 2 tumors in 4 of the females, and as many as 6 separate tumors were observed in 1 female before it died. A total of 16 tumors developed in 7 females of the first group of 13, an incidence of 54 percent. All of these tumors were histologically adenocarcinoma of the breast except 1 of the 6 tumors which had developed in 1 animal. This was found to be an adenomatous mammary tumor with mucoid change in the glands and stroma and was classified as possibly malignant. In contrast only 1 of the 5 males developed a visible tumor. This was located behind the left ear and histologically proved to be a squamous-cell carcinoma with osseous metaplasia. The first type B tumor was found by palpation in the course of routine examination in front of the left hind leg of a male rat after 9 months of treatment with methylcholanthrene. There was no striking increase in size of the lesion in the following 3 weeks of observation. On histologic examination it was diagnosed as an adenocarcinoma in hyperplastic breast tissue.

A second group (Group 2), consisting of 15 female and 9 male rats, was subjected to the identical experimental conditions as in Group 1, in order to determine the reproducibility of our initial results. One female of this group died early in the experiment. The first adenocarcinoma of the breast (type A) appeared in a female rat 143 days after methylcholanthrene was started, and the last tumor was detected in a female rat at 266 days. Twelve tumors, all adenocarcinoma of the breast, developed in 7 (50 percent) of the 14 females in this group up to the date when observations for this report were ended (April 1, 1949). Single breast cancers appeared in 5, 2 in 1, and 5 in 1 of the female rats.

Two obvious questions arise from the results in these two groups: (1) Did the calcium-deficient diet contribute to the production of the breast lesions; and (2) is there an ovarian factor involved? Accordingly, one group of animals on a complete diet (Group 3) and another castrated group (Group 4) were placed under treatment. Group 3, comprised of 10 females and 5 males, was treated like Groups 1 and 2 except that these animals were maintained on our colony Rockland rat diet throughout the

experimental period. Methylcholanthrene treatment was started on April 22, 1948. The first tumor (type A) appeared in a female rat at 133 days, and thus far a total of 14 type A tumors have appeared in 7 of the females in this group. Histologically these have all proved to be breast adenocarcinoma. In 3 animals we found single tumors; in 3 others, 2 tumors in each; and in 1 rat 5 separate breast cancers have developed.

In addition, in four of the females in Group 3, type B tumors appeared in the skin folds. Three occurred in females in which no other tumor was observed, while one developed in a female in addition to a type A tumor, which histologically also proved to be an adenocarcinoma of breast tissue. The remaining type B tumors have not been removed in order to permit observation during a longer period of development. Thus far the incidence of identified adenocarcinoma of the breast in this group of females is 70 percent.

A tumor classified grossly as type A appeared for the first time in a male animal in Group 3, 129 days after the experiment was started. The tumor grew at a moderately rapid rate, was excised 2 weeks after its first appearence, and weighed 7 gm. Histologically the lesion was classified as arising from breast tissue and showed areas considered to be possibly malignant. Sixty-seven days after its removal, a second tumor appeared in the same region. This grew rapidly and when removed 13 weeks later, the tumor weighed 117 gm. and measured $8.0 \times 4.6 \times 4.2$ cm. Histologically this lesion was an adenocarcinoma of the breast. The animal, including the tumor, weighed 326 gm.

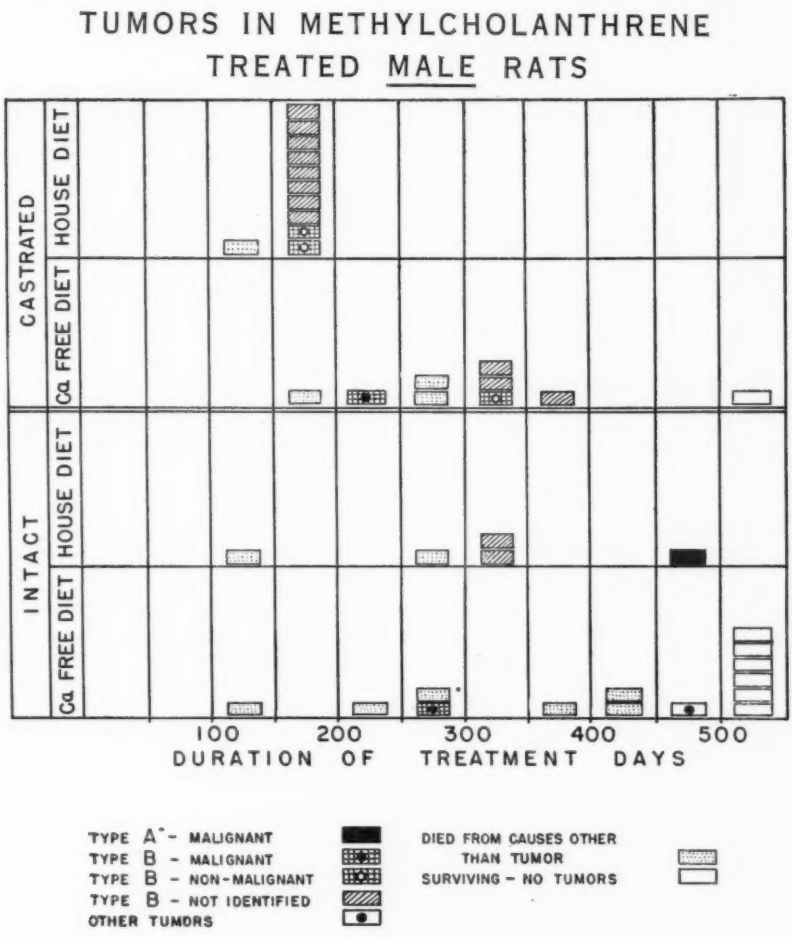
Type B tumors which have not yet been removed for histologic study have been found in two other males in this group.

The animals in Group 4, comprising 15 females and 22 males, were castrated at the beginning of the experiment. The females were all treated exactly as in Group 1. Methylcholanthrene administration was started in the castrated females on April 13, 1948. As of April 1, 1949, 9 animals in this group have died; 2 of them died before 6 months of treatment and should perhaps be excluded from the group. None of the females has thus far developed a type A breast tumor although 3 show tumors of type B, the exact histologic structure of which remains to be determined.

Of the 22 castrated males currently under study, 10 have been maintained on the calcium-free diet administered as outlined earlier in this report, while 12 are on the Rockland diet. Gastric instillation of methylcholanthrene was started on the group of 10 on April 2, 1948, and on the group of 12 on September 9, 1948. It is interesting to note that, although treatment with the carcinogen in the animals on the Rockland diet was started more than 5 months later than in those on the calcium-free diet, up to the present time tumors of type B have appeared in 10 of the former and in only 5 of the latter group. A tumor in 1 of the 5 has been removed

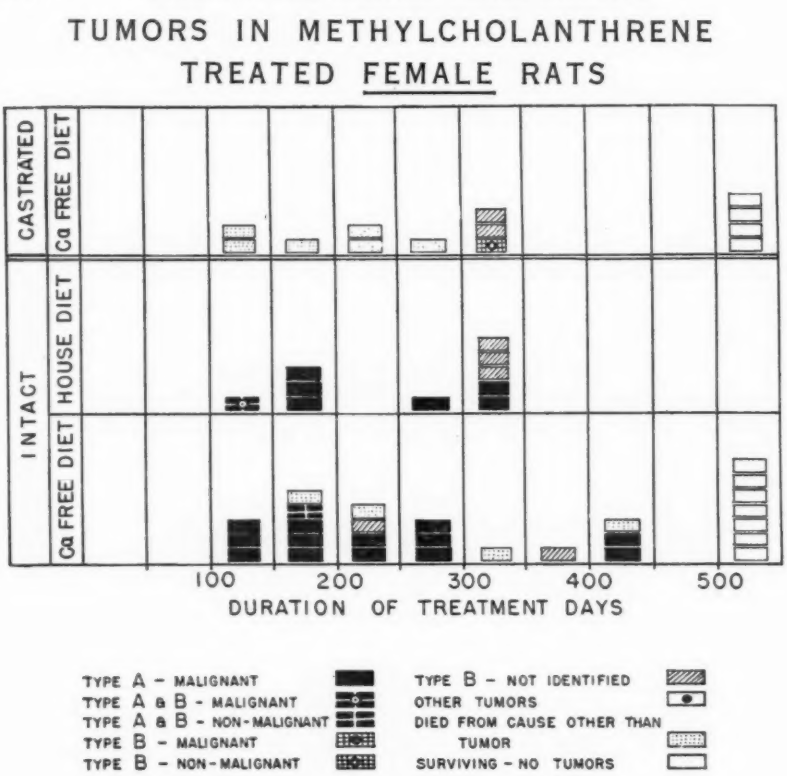
and histologically is an adenocarcinoma arising in hyperplastic breast tissue.

Cystic masses have developed at the site of castration in some of the male animals on either diet. These have been excised, and histologic



TEXT-FIGURE 1.

examination has shown the walls of the cysts to consist of dense fibrous scar tissue lined in part by squamous epithelium. The cavities contain a suppurative exudate and show no evidence of any neoplastic change. In text-figures 1 and 2 all animals used for this report have been combined to show the distribution, type of tumor, and approximate appearance date of the first tumor in the male and female animals.



TEXT-FIGURE 2.

DISCUSSION

In the introduction, we indicated that the primary purpose of this investigation was to produce gastric glandular carcinoma by the local instillation of a carcinogen. Our efforts, like those of previous investigators, failed in its original objective. Although several carcinogens have been administered orally by others (*8–12*), we have limited our consideration of the literature to those investigations in which methylcholanthrene was the carcinogen employed. Bile regurgitated into the stomach has been considered a possible source of a contributory agent in the genesis of gastric cancer (*13*). Since methylcholanthrene is chemically related to bile acids, it would be the logical carcinogen to try in experiments planned to induce gastric cancer. Van Prohaska, Brunschwig, and Wilson (*14*) in 1939 fed mice this agent in olive oil with a glass dropper. The largest and most sustained experiment involving the feeding of a carcinogen has been carried out in mice by Lorenz and Stewart (*15*), who administered methylcholanthrene as an emulsion in the drinking water. Ingestion by the mice of such emulsions made up with olive oil

resulted in a high incidence of carcinoma of the small intestine. In contrast, carcinoma of the forestomach rarely developed. However, when the olive-oil emulsions were made acid-stable by the addition of a wetting agent, squamous carcinoma of the forestomach developed with increasing frequency (*15*). Our method of administration of the carcinogen (methylcholanthrene) more closely resembled the nonacid-stable form in olive oil used by Lorenz and Stewart (*15*). While the amounts of carcinogen administered to our rats was comparable with those fed to mice by Lorenz and Stewart, the striking species difference in response is of interest in that not a single gastrointestinal tumor appeared in our animals.

Stewart and Lorenz (*16*) have produced gastric glandular carcinoma in mice by the direct injection of methylcholanthrene into the stomach wall. Recently Howes and de Oliveira (*17*) reported similar lesions in Wistar rats following the implantation of methylcholanthrene into the wall of the stomach by means of suture threads impregnated with the carcinogen.

The high incidence of adenocarcinoma of the breast developing as a result of the intragastric instillation of methylcholanthrene is especially significant since the spontaneous development of cancer of the breast is rare in the rat, particularly during the age periods in which our experiments were conducted.

Bullock and Curtis (*18*) in autopsies of more than 2,450 rats found 521 spontaneous tumors in 489 animals. Of this group, 94 were unquestionably breast tumors; all except 7 were benign lesions. Only 4 of the malignant lesions were epithelial in origin and appeared at 337, 354, 544, and 624 days, respectively.

In the post mortem examination of 216 rats used in an experiment designed to study the nutritional requirements of the rat in the latter half of its life, Saxton and Kimball (*19*) found 51 tumors in 48 animals, none of which were breast tumors. Similarly Nelson and Norris (*20*) found only 2 benign breast tumors in 323 albino rats autopsied at 645 to 790 days of age.

More recently Keston (*21*), in autopsy studies of the older rats of the Wistar Institute colonies, detected tumors in 164 of 630 rats examined. The mean age of these animals was 810 days (standard deviation 210 days). None of these tumors, however, proved to be an adenocarcinoma of the breast.

A comparison of the results obtained by Lorenz and Stewart (*15*) in mice with our own in Wistar rats again indicates a striking difference in species response. In the large number of feeding experiments with methylcholanthrene in mice, Lorenz and Stewart failed to find a single adenocarcinoma of the breast. Van Prohaska, Brunschwig, and Wilson (*14*) have reported the development of adenocarcinoma of the breast in 4 of 15 white mice, following the oral administration by glass dropper of methylcholanthrene in olive oil. These investigators, however, point out that all mammary tumors arose well forward on the thorax, an area over which the oil and carcinogen spread during each intraoral application.

No mammary cancer was observed in 33 animals to which the methylcholanthrene was administered in lard.

Group 3, in which the animals were maintained on the Rockland rat diet throughout the experiment, was especially pertinent to our problem since Suntzeff and Carruthers (*22,23*) have shown that there is 50 percent less total calcium in methylcholanthrene-induced epidermal hyperplasia than in normal epidermis. In squamous-cell carcinoma they found a still greater reduction in calcium content which was approximately 60 percent of that present in precancerous hyperplastic epidermis. Brunschwig, Dunham, and Nichols (*24*) also observed a sharp drop in calcium in cancer tissue, and Lansing (*25*) found that the ultra-filterable calcium fraction of a squamous-cell carcinoma was considerably lower than hyperplastic mouse epidermis. Recently Coman (*26*) reported that normal cells in a calcium-free medium are more readily separated by micromanipulation than those in balanced salt solution, and he demonstrated further that cancer cells are more readily separated than are normal cells. He suggested that the decreased adhesiveness of cancer cells may result from a local calcium deficiency which facilitates their separation.

The results in Group 3 are particularly significant since the incidence of adenocarcinoma of the breast was as great or greater in the animals on the colony diet as in those maintained largely on the calcium-free diet. These findings indicate, therefore, that the local changes in the calcium content of malignant tissue are not related to the level of calcium in the diet and that the deficient calcium diet did not contribute to the development of the breast cancers in our animals.

The purpose in studying the effect of castration in females (Group 4) needs little discussion. Long before the identification of estrone, the function of the ovaries was thought to be an important factor in the development of mammary cancer. This view appeared to be confirmed in the laboratory by Loeb and by Cori (*28*) in castration experiments; by Murray (*29*) and DeJongh and Korteweg (*30*) by the transplantation of ovaries into spayed males. However, the results of Woolley (*31*) obtained in female mice in parabiosis and those of Lacassagne and Raynaud (*32*) following treatment of female mice with testosterone propionate, indicate that the development of the breast, which is dependent in part on ovarian function, is the determining influence in the appearance of experimental breast cancer and not of the ovarian secretion per se.

At this time we are unable to define exactly the role of ovarian secretion in the development of breast cancer in our experiments. To date we have observed for a sufficiently long period only a group which was castrated at 5 to 6 weeks of age. A striking decrease in incidence of breast cancer was noted. But in evaluating this fact, one must take into consideration a possible failure of adequate breast development in the animals castrated at so early an age. Studies are currently under way to determine the effect of castration in females shortly after reaching sexual maturity, as well as the influence of estrogen implants on the effect of methycholanthrene administered in young castrates.

The castrated males were included in the study in order to determine whether castration would disturb the androgen-estrogen ratio sufficiently to bring about the development of breast cancer in the males receiving methylcholanthrene. It is quite possible that the production of estrogen in the male castrate rat is entirely a function of the adrenal glands, since very little or no estrogenic material is produced in organs other than the ovaries, testes, placenta, and adrenals (*33*). After castration of newborn dba male mice, Woolley, Fekete, and Little (*34*) observed hypertrophy of the adrenal cortex, and in many of these animals they noted development of the mammary glands. Cancer of the breast appeared in one of these male mice. It would seem that castration might provide more favorable conditions for the development of breast cancer following the administration of methylcholanthrene than those which exist in the intact male. Our results indicate that some derangement of the sex hormone balance did occur in the male castrates to bring about changes in the breast tissue. A number of the type B tumors which developed in this group showed hyperplasia of breast tissue and cancer formation; others proved to be hyperplastic breast tissue some with areas of atypical mammary glands.

Methylcholanthrene may also produce some estrogenic effect. Cook and Dodds (*35*) demonstrated many years ago an estrus-exciting activity in the two most potent carcinogenic hydrocarbons then extant, namely, 5,6-cyclopenteno, 1,2-benzanthracene, and 1,2-benzpyrene. Administered for long periods to rats, methylcholanthrene has a toxic effect on the bone marrow and produces changes very similar to those described recently by Crafts (*36*) following large doses of estrogen in dogs. The changes observed in the bone marrow are also similar to those found by Lorenz and Stewart (*37*) in mice, following prolonged administration of dibenzanthracene. These investigators observed a variable degree of depletion of the bone marrow in animals receiving dibenzanthracene in the drinking water for approximately 5 months. In some of our rats this depletion became so severe that only a few cells were found in the bone marrow smear, a final picture similar to that described by Crafts (*36*).

SUMMARY AND CONCLUSIONS

The intragastric instillation of methylcholanthrene into the stomach of Wistar rats for many months failed to produce any gastric or intestinal lesions, in spite of the fact that ulceration of the antrum must have been present for part of this period in some of the animals.

The gastric instillation of methylcholanthrene resulted in a very high incidence (50–70 percent) of adenocarcinoma of the breast in noncastrated females.

Sponataneous adenocarcinoma of the breast in the Wistar rat has not been reported during the age period in which our experiments were conducted.

Thus far proven adenocarcinoma of the breast has appeared in only 2 noncastrated males in a group of 19 and in 1 castrated male in a group of 20.

Our results indicate that methylcholanthrene may have some estrogenic activity in addition to its carcinogenic action.

Methylcholanthrene instilled into the stomach is absorbed from the intestine and it or its degradation products reaches the mammary glands and there exerts a carcinogenic effect and possibly some estrogenic action also.

The incidence of the breast cancer in the noncastrated female and the sharp decrease in this incidence in the female castrated early in life indicate that ovarian secretion plays an important contributory role in the development of the breast lesions. Whether this secretion is important only as it contributes to the adequate development of the breast or whether it serves as a cocarcinogen cannot be stated at the present time.

ADDENDUM

Since observations were closed for the purpose of this report, the remaining three females in the group maintained on the colony (Rockland) diet developed an adenocarcinoma of the breast (type A tumor). Histologically these tumors were identical with those which appeared in the other animals, thus making a 100-percent incidence of breast cancer in the group of females which were fed the colony diet. The last tumor was detected in this group at 383 days.

Although the induction time for breast tumors described in this paper, when compared with that for transplantable tumors, is relatively long, nevertheless we think that such tumors would have merit for a screening program in the chemotherapy and perhaps also in the chemoprophylaxis of cancer. Stock and Rhoads (*38*) have reported recently that the use of transplantable growths in a screening program is open to many theoretical and a few practical objections, and they have placed the value of such tumors in the uniformity of the test material that they provide. The breast cancers induced in our animals have shown a remarkable uniformity of structure. Even more suggestive than for use in a chemotherapeutic screening program is the possible application of our experimental approach to a screening program of chemoprophylactic agents in cancer. The breast lesions induced after the gastric instillation of a carcinogen which theoretically can be derived from a normal constituent of the body economy, permit some speculation as to whether the mechanism for the production of these cancers might not closely resemble the mechanism of spontaneous cancer. The only difference seems to be that we can identify the trigger substance (methylcholanthrene) in our rats, while we are not aware of the trigger substance in spontaneous cancer. Such reasoning, if followed through, leads naturally to the possible use of these induced cancers to study the biological alterations that precede the actual appearance of the cancer, especially since we know now that it is not necessary to continue the gastric instillation of methylcholanthrene until the cancer appears,but that the administration of the carcinogen may be stopped after a time and then sometime later the breast cancer may appear. From a chemo-

prophylactic standpoint, animals treated as described may possibly serve as a tool in the search for anticarcinogenic chemicals.

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PRELIMINARY STUDIES OF THE EFFECTIVENESS OF LOCAL IRRADIATION IN THE INDUCTION OF LYMPHOID TUMORS IN MICE[1,2]

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Induction of lymphoid tumors following whole-body exposure of mice to ionizing radiation has been reported by a number of investigators (*1–4*). Evidence has been adduced that, in some strains at least, the thymus gland is the site of predilection for the development of these tumors (*5, 6*). The mechanism of their induction is still a subject of conjecture. As a working hypothesis, the incident radiation may be considered to act locally and directly upon the cell or tissue of origin to produce a neoplastic alteration in cellular behavior. If this were true, it would be unnecessary to irradiate the whole body; it should be possible to obtain a comparable incidence of lymphoid tumors by exposure sharply localized over a susceptible region. This paper presents the results of three related experiments intended to test this hypothesis.

EXPERIMENT 1

Strain A (Strong) mice 2 months of age were immobilized and irradiated through a circular portal 1 cm. in diameter over one of four dorsal regions: the skull, the mediastinum, the lumbar region, and the base of the tail.[3] The four groups were identically caged and maintained on Purina laboratory chow and water ad libitum until they became moribund or died. All animals were autopsied, and abnormal gross findings were checked by histologic examination of the appropriate tissues.

The results are presented in table 1. With the exception of animals which died soon after irradiation, most of the mice survived 12 to 18 months. No lymphoid tumors were observed in any of the four groups. In previous experiments with mice of this strain, fractionated whole-body radiation in doses totalling 600–750 r has yielded a lymphoma incidence ranging from 10 to 30 percent.

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[2] These investigations were initiated in the Department of Radiology, Yale University School of Medicine, and have been continued and extended in the Biophysics Section, National Cancer Institute, Public Health Service, and the Department of Radiology, Stanford University School of Medicine, with the aid, in part, of grants from the Jane Coffin Childs Memorial Fund for Medical Research and from the National Cancer Institute.

[3] Physical factors were: 100 KVP, 6 ma., focus-skin distance 17.8 cm., 2 mm. Al added filter, output 88 r per minute, dose 176 r per day on 12 consecutive days for a total dose of 2112 r. The untreated areas were carefully shielded with lead.

TABLE 1.—*Tumor incidence following regional irradiation of strain A mice*

Region treated	Mice								
	Number treated			Number surviving treatment			Number having lymphoid tumors		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
Skull	15	14	29	11	9	20	0	0	0
Mediastinum	19	15	34	18	10	28	0	0	0
Lumbar	11	20	31	10	18	28	0	0	0
Base of tail	15	16	31	11	9	20	0	0	0

EXPERIMENT 2

It was felt that the negative results of Experiment 1 might be related to the low susceptibility of strain A mice to radiation-induced lymphomas. Accordingly, animals of the C57 black strain, which has been shown to be highly susceptible to lymphoma induction following whole-body irradiation (*7*, *8*), were selected for the next experiment. Sixty mice, 6 to 8 weeks of age and of both sexes,[4] were divided into two equal groups, immobilized, and exposed to a single roentgen treatment of 1,000 r[5] delivered to a transverse body section, 4 mm. in thickness, at the level of the mediastinum or upper abdomen, respectively. The animals were identically caged and maintained as in Experiment 1. After an observation period of 16 months, no lymphoid tumors were noted in either group.

EXPERIMENT 3

Because the treated volumes in the above experiments were quite small, it seemed of interest to irradiate a larger but nevertheless localized region. Strain C57 black mice of both sexes [4] were again used and irradiation started at 1 month of age. A total of 1,000 r was delivered in 10 treatments (5 daily treatments in 2 consecutive weeks).[5] Sixty-nine mice were treated over the upper (cranial) half and 66 mice over the lower (caudal) half of the body.

As a result of post-irradiation deaths and other early losses, the number of determinate mice diminished to 55 in each group. After 18 months, only 3 lymphoid tumors (3 percent) were observed: 2 in the group treated over the upper, and 1 among those treated over the lower half of the body.

For ease of comparison and to facilitate discussion, these results are contrasted in table 2 with the incidence of lymphoid tumors observed in this laboratory after various doses of whole-body radiation.

[4] Previous studies (unpublished) with mice of this strain have established that the lymphoid tumor response after irradiation at 1 month of age does not differ significantly for the two sexes.

[5] Physical factors were: 186 KVP., 20 ma., 0.25 mm. Cu and 0.55 mm. Al filter. Focus-skin distance in Experiment 2 was 30 cm., with an output of 180 r/min.; in Experiment 3, 47 cm. with an output of 66 r/min. Lead shielding was carefully placed to outline the treatment area.

TABLE 2.—*Comparative lymphoid-tumor incidence after whole- and half-body irradiation*

Strain [1]	Part of body exposed	Treatments	Total dose	Mice surviving irradiation	Lymphoid tumors	
		Number	*r*	*Net number*	*Number*	*Percent*
C57 black	Upper half	10	1,000	55	2	4
C57 black	Lower half	10	1,000	55	1	2
C57 black	Whole	12	1,000	58	37	64
C57 black	do	10	750	46	22	48
Strong A	do	12	600	31	9	29

[1] All groups of mice were 1 month old at the time of irradiation. Although the experiments were not all done at the same time, all mice of both strains were bred in the laboratory from the same original breeders, generously supplied by Dr. L. C. Strong, Department of Anatomy, Yale University School of Medicine.

DISCUSSION

Localized irradiation of mice of two different strains has failed to yield lymphoid tumors with a frequency comparable to that observed after whole-body exposure. Evaluation is complicated by the lack of a basis for comparison of dosage in the two methods of irradiation.

From the originally stated working hypothesis, it is to be expected that roughly equal doses per unit volume delivered at a susceptible site should yield an equivalent number of lymphoid tumors. This condition is apparently met in Experiment 3, where the dose per unit volume is almost identical with that administered in previous whole-body irradiation experiments involving mice of the same strain and age. Despite this, there is a profound discrepancy in tumor incidence as indicated in table 2. It is difficult to escape the conclusion that the induction of mouse lymphoid tumors by irradiation is not solely the result of a direct, local effect upon a susceptible cell or tissue.

These negative results in themselves, of course, do not establish the existence or nature of an indirect mechanism; yet they, together with a number of other considerations, suggest that systemic responses may be interposed between the initial exogenous stimulus of irradiation and the ultimate development of lymphoid tumors. Susceptibility to lymphoid-tumor induction has been shown to vary with such factors as age and strain (*3*, *7*, *8*, *9*). While these effects might conceivably be attributed to intrinsic differences in the lymphocytes or their precursor cells, it would seem more plausible to associate them with genetic and age-dependent variations in certain physiologic states which may play an intermediate role in the induction process.

Reference has already been made to the observation that in some strains of mice, including those studied in the present experiments, virtually all radiation-induced lymphomas appear to originate in the thymus. If their development were the result of the direct action of radiation upon lymphoid tissue, it might be expected that the site of origin would vary among the lymph nodes, thymus, spleen, and other lymphoid foci in rough proportion to their respective weights or lymphoid tissue content. The rather highly selective thymic response in this instance is not unique;

a similarly disproportionate effect upon the thymus has been observed following another type of involutional stimulus: the injection of pituitary adrenocorticotrophic hormone or of whole adrenal cortical extract (*10, 11*).

Finally, Lorenz and Eschenbrenner [6] have observed the development of lymphomas in strain A mice irradiated over the entire body with the thorax (and thymus) shielded. The incidence was significantly increased but somewhat less than that noted with whole-body irradiation. No lymphomas were found in mice treated over the thorax alone. Further studies are needed to trace the histogenesis of such tumors. If the thymus should prove again to be the site of origin, an indirect induction mechanism would clearly be implicated, since the thymus is not included in the field of irradiation with this technique.

SUMMARY

In sharp contrast to previous observations following whole-body exposure, local roentgen irradiation of mice of strains A and C57 black, administered somewhat differently in three related experiments, consistently failed to yield lymphoid tumors with significant frequency. It is concluded that the induction of these tumors is probably not solely the result of a simple direct action of radiation upon the susceptible cell or tissue of origin.

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THE NEOPLASTIC TRANSFORMATION AS A BIOLOGICAL FRACTIONATION OF RELATED ENZYME SYSTEMS [1]

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Extensive studies have shown that when a normal tissue becomes cancerous certain enzyme systems increase in activity, others decrease, while still others undergo relatively little change (cf. *1*). For the most part, these observations have been concerned with a wide variety of intracellular catalytic systems which bear apparently little metabolic relation with each other. Thus, although it is desirable and important to know that certain oxidase systems decrease with the neoplastic transformation of a normal tissue, while simultaneously certain proteolytic capacities may increase, the very lack of present information in respect to the possible physiological relation between oxidation and proteolysis renders such observations largely empirical in character. It may be taken for granted that in the highly integrated metabolic mosaic of the living tissue no catalytic system functions completely independently of every other system, but, except for such groups of activities as are concerned with the glycolytic and citric acid cycles, relatively little is known about the metabolic interrelationships among enzyme systems acting upon substrates of widely varying constitution by mechanisms often differing greatly in character. This lack of information has handicapped attempts at a rationalization of the considerable enzymatic data accumulated on the neoplastic transformation, and little can be done in this respect at the present time except to encourage intensive fundamental investigations on the metabolism of normal tissues.

It would be absurd, however, for the tumor enzymologist to postpone in despair his investigations on cancer tissues until the nature of all of the enzymatic mechanisms of normal tissue is revealed. It is rather within his province to suggest, from his own experience with cancer tissues, those fundamental investigations on normal tissues which might conceivably be helpful in the interpretation and rationalization of results on cancer tissues. In this way, a frequently mutual helpfulness may be achieved, whereby the fields of normal and of pathological biochemistry are equally benefited. Encouraging in this respect is the fact that the changes in the level of metabolic systems which accompany the neoplastic transformation are not capricious and chanceful, but appear to follow a definite pattern (cf. *1*, pp. 198–202).

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In view of these considerations, it seemed desirable to lay aside for the time being our broad survey of the catalytic systems of tumors, and, instead, to concentrate upon a few enzymes of special interest. These enzymes have been selected because they act upon substrates similar, analogous, or isomeric in chemical constitution, and in the study of the changes which such systems undergo in the neoplastic transformation we are enabled to focus our attention on systems which may be assumed to be metabolically related. Such enzymes are those which act upon the -C-N- bond, namely, amidases, dehydropeptidases, and peptidases. The comparison tissues studied have been normal rat liver and the primary hepatoma induced by the feeding of *p*-dimethylaminoazobenzene on the one hand [3] and normal mouse liver and the transplanted hepatomas 112B and 98/15 on the other. In some instances, samples of fetal liver were included in these studies.

A wide variety of these enzymes exist in all normal tissues. In any one tissue, certain of these systems may be separated from others of closely similar function by such means as differential centrifugation of the tissue extract or homogenate, or selective denaturation by heat, or other means. As a result, a chemical fractionation of these activities is achieved whereby one or more systems are concentrated while others may be expeditiously removed. Such a chemical fractionation may, in effect, be imitated by the biological fractionation inherent in the neoplastic transformation of a normal tissue. Examples of such fractionations follow, with data drawn partly from the literature, and partly from new experiments.

ω-Amidases

At least two glutaminases and two asparaginases exist in rat and mouse liver (*2–4*). Glutaminase I is sedimentable on high speed centrifugation of liver homogenates, is relatively heat-labile and acid-labile, and is activated by such anions as phosphate, arsenate, and sulfate, but not by pyruvate. Glutaminase II is a soluble enzyme, relatively heat- and acid-stable, and is active only in the presence of added pyruvate or other α-keto acid, but not of phosphate, arsenate, or sulfate. Asparaginase I is a soluble enzyme, highly active in crude liver homogenates, acid-labile and heat-labile, and activated by a number of salts. Asparaginase II is also a soluble enzyme, relatively acid-stable and heat-stable, activated only by pyruvate or other α-keto acid, and measurable only in heated homogenates of liver in which asparaginase I activity is destroyed. Glutaminase I and asparaginase I are found in all animal tissues studied, both normal and neoplastic. Glutaminase II and asparaginase II are found only in liver and hepatoma, the tumor derived from liver.

Data on the optimal rates of ω-amidase activity in various rat tissues are given in table 1. Part of these data are derived from the paper by Errera and Greenstein (*5*), the remainder represent new experiments under conditions identical with those described in the reference mentioned.

[3] Donated by Dr. J. White.

TABLE 1.—*ω-Amidase activity in rat hepatic tissues* [1]

Enzyme	Micromoles substrate × 100 desamidated per hour per mg. N in:		
	Adult liver	Hepatoma	Fetal liver
Glutaminase (inherent)[2]	2	20	20
Glutaminase I (optimal phosphate)	166	1, 000	1, 300
Glutaminase II (optimal pyruvate)	48	28	32
Asparaginase (inherent)[2]	36	16	13
Asparaginase II (optimal pyruvate)	50	18	22

[1] Digests consisted of 1 cc. aqueous tissue extract, 2 cc. 0.018M veronal buffer alone or containing either 0.2M phosphate or 0.06M pyruvate so as to give pH 7.8, and 1 cc. of either water or 0.014M amide. Activity measured in terms of ammonia evolved (*2-4*).
[2] Values in aqueous extract without any added phosphate or pyruvate.

Similar data involving the amidase activity of mouse hepatic tissues are given in table 2.

TABLE 2.—*ω-Amidase activity in mouse liver and hepatomas 98/15 and 122B* [1]

Enzyme	Micromoles substrate × 100 desamidated per hour per mg. N in:		
	Adult liver	Hepatoma 98/15	Hepatoma 112B
Glutaminase (inherent)[2]	2	20	24
Glutaminase I (optimal phosphate)	160	600	500
Glutaminase II (optimal pyruvate)	48	38	40
Asparaginase (inherent)[2]	32	12	----
Asparaginase II (optimal pyruvate)	60	20	----

[1] Experimental conditions as in table 1.
[2] No added phosphate or pyruvate.

In the course of neoplasias of liver, the so-called inherent glutaminase activity of liver increases, that of inherent asparaginase activity decreases. Glutaminase I activity considerably increases, glutaminase II is appreciably decreased, while asparaginase II considerably decreases. In all cases, the relation of activities of fetal liver to adult liver resembles that of hepatoma to adult liver. There is little doubt that a marked fractionation of the glutaminase and asparaginase activities (except for glutaminase II) had occurred in the course of the neoplastic transformation of liver. Even if it were not for the chemical fractionation of liver alone, whereby glutaminase was separated from asparaginase, and the different glutaminase and asparaginases from each other, it would be amply clear that we were dealing with several individual enzyme systems.

The possession by the hepatoma of glutaminase II activity is very probably a retention of some specfiic activity of the tissue of origin, since no other kind of tumor studied possesses a trace of this enzyme (*5*). This is a situation not uncommonly encountered in oncology, for certain thyroid

tumors, islet tumors, etc., may still secrete the respective hormones. It will be noted, however, that the hepatoma contains less glutaminase II activity than normal liver.

DEHYDROPEPTIDASES

There are at least two classes of dehydropeptidases, namely (1) those which act upon dehydropeptides with a free α-amino group adjacent to the susceptible dehydropeptide bond, and (2) those which act upon dehydropeptides which lack this free α-amino group (*6*). The former group, characterized by the designation of dehydropeptidase I is widespread and has been found to occur in all animal tissues studied, both normal and neoplastic, as well as in all plant tissues studied. The latter group, designated dehydropeptidase II, has a more limited distribution and is found only in kidney, liver, and pancreas of animal tissues and in a few plant tissues (*6*). As representatives for each of the groups, glycyldehydroalanine and chloroacetyldehydroalanine respectively, may be cited.

Dehydropeptidase I has been separated from dehydropeptidase II in hog kidney homogenates by differential centrifugation (*7*). This fractionation is also achieved by the neoplastic transformation of rat liver, for, as noted in table 3, while liver possesses both dehydropeptidases I and II, the hepatoma possesses only dehydropeptidase I. In the course of the neoplastic transformation of liver (and presumably of other normal tissues), dehydropeptidase I has become concentrated in activity, and dehydropeptidase II has been eliminated. So far as we know at the present time, this is a general phenomenon (cf. *8*). But this is not all of the fractionation which the neoplastic transformation effects in this class of enzymes.

Dehydropeptidase I in kidney, liver, etc., acts upon a variety of substrates containing a free α-NH_2 group adjacent to the dehydropeptide bond, such as glycyldehydroalanine, glycyldehydrophenylalanine, N-methylglycyldehydroalanine, and DL-alanyldehydroalanine. When the liver undergoes a neoplastic transformation, however, the activity in the resulting hepatoma toward glycyldehydroalanine and DL-alanyldehydroalanine increases, whereas that toward N-methylglycyldehydroalanine diminishes, and that toward glycyldehydrophenylalanine disappears (table 3). It seems highly probable, therefore, that the enzyme termed dehydropeptidase I is multiple in character and that the neoplastic transformation effects a fractionation of the subelements of this enzyme system whereby that system or systems concerned with the hydrolysis of glycyldehydroalanine and DL-alanyldehydroalanine are concentrated whereas that concerned with the hydrolysis of glycyldehydrophenylalanine is eliminated (table 2). Again, this appears to be a general phenomenon (*8*, *9*).

In the case of the tripeptides, such as glycylglycyldehydroalanine, the acyl peptide bond is probably rapidly hydrolyzed, leaving the susceptible dehydropeptide. Of the series of α,α-di(acylamino)propionic acids studied,

only the glycyl compound increases in susceptibility in the course of the neoplastic transformation. The capacity to hydrolyze the other compounds of this series disappears. Again there is a concentration in activity toward one member of the series and a disappearance of activity toward others.

TABLE 3.—*Susceptibility to enzymatic hydrolysis by rat hepatic tissues of a variety of dehydropeptides and related compounds* [1]

Substrate	Micromoles × 10 ammonia N evolved per mg. total N in extract of—	
	Liver	Primary hepatoma
Acetyldehydroalanine	3	0
Chloroacetyldehydroalanine	28	0
Glycyldehydroalanine	60	384
Chloroacetylglycyldehydroalanine	2	0
DL-Chloropropionyldehydroalanine	8	0
DL-Alanyldehydroalanine	152	1, 152
Chloroacetyl-DL-alanyldehydroalanine	26	21
Acetyldehydrophenylalanine	0	0
Chloroactyldehydrophenylalanine	0	0
Glycyldehydrophenylalanine	12	0
Chloroacetylglycyldehydrophenylalanine	0	0
Acetyldehydroaminobutyric acid	0	0
Acetyldehydrovaline	0	0
Acetyldehydronorleucine	0	0
Acetyldehydroleucine	0	0
Acetyldehydrotyrosine	0	0
N-Methylglycyldehydroalanine	25	4
Chloroacetyl-N methylglycyldehydroalanine	0	0
Glycylglycyldehydroalanine	60	372
N-Methylglycyldehydrophenylalanine	6	0
DL-Alanyldehydrophenylalanine	8	0
N-Methyl-DL-alanyldehydrophenylalanine	5	0
DL-Chloropropionyldehydrophenylalanine	0	0
α, α-Di(Glycylamino) propionic acid	2	4
α, α-Di(N-methylglycylamino) propionic acid	1	0
α, α-Di(N-dimethylglycylamino) propionic acid	0	0
α, α-Di(Chloroacetylglycylamino) propionic acid	0	0
α-(DL-Chloropropionylamino)-α-(DL-alanylamino) propionic acid	0. 6	0
α, α-Di(DL-alanylamino) propionic acid	0. 6	0

[1] Digests consisted of 1 cc. aqueous tissue extract, 2 cc. 0.10M borate buffer at pH 8.1, and 1 cc. of either water or 0.025M neutralized substrate solution. With racemic substrates, a 0.050M solution was used.

PEPTIDASES

Like the dehydropeptides, chloroacetyldehydroalanine and glycyldehydroalanine, which are acted upon by individual enzymes, the analogous, saturated peptides, chloroacetyl-L-alanine and glycyl-L-alanine are acted upon by different enzymes (*7*). As a result of the neoplastic transformation of liver to hepatoma, dehydropeptidase-II activity disappeared. In this same transformation, however, the capacity to hydrolyze a variety of chloroacetylated-L-amino acids is virtually unchanged (table 4). It would appear from these data, as well as from other studies (*7*, *10*), that

all of the substrates listed in table 4 may well be attacked by a single enzyme system.

TABLE 4.—*Enzymatic hydrolysis of N-acetyl amino acids with rat tissues* [1]

Substrate	Micromoles substrate hydrolyzed per hour per mg. N in:	
	Liver	Primary hepatoma
Acetylglycine	0	0
Acetyl-DL-alanine	30	23
Acetyl-DL-valine	30	25
Acetyl-DL-leucine	35	33
Acetyl-DL-methionine	70	67
Acetyl-DL-histidine	0	0
Acetyl-DL-glutamic acid	41	42

[1] Digests consisted of 1 cc. aqueous tissue extract, 1 cc. phosphate buffer at pH 7.1, and 1 cc. of either water or 0.050M neutralized substrate. Activity measured by ninhydrin, CO_2 manometric procedure.

A more complicated picture arises in the case of substrates with a free α-amino group adjacent to the susceptible peptide bond (table 5). The neoplastic transformation results in a decrease in activity toward glycylalanine, glycylphenylalanine, leucylglycine, and phenylalanylglycine, with a concomitant rise in activity toward alanylglycine and glycylleucine (cf. *11*).

TABLE 5.—*Hydrolysis of isomeric peptides in rat tissues* [1]

Substrate	Micromoles substrate hydrolyzed per hour per mg. N in:	
	Liver	Hepatoma
Glycyl-DL-alanine (L)	2, 000	1, 200
DL-Alanylglycine (L)	1, 630	3, 700
Glycyl-DL-leucine (L)	2, 970	7, 475
DL-Leucylglycine (L)	1, 055	72
Glycyl-DL-phenylalanine (L)	3, 200	2, 120
DL-Phenylalanylglycine (L)	520	57

[1] Digests and experimental conditions as in table 4.

In the case of mouse tissues, the neoplastic transformation of liver to the transplanted hepatoma 112B is accompanied by a concentration in activity toward all the peptides studied (table 6).

TABLE 6.—*Hydrolysis of isomeric peptides in mouse tissues* [1]

Substrate	Micromoles substrate hydrolyzed per hour per mg. N in:	
	Liver	Hepatoma 112B
Glycyl-DL-alanine (L)	920	4, 520
DL-Alanylglycine (L)	1, 340	6, 060
Glycyl-DL-leucine (L)	4, 230	7, 240
DL-Leucylglycine (L)	580	2, 580
Glycyl-DL-phenylalanine (L)	2, 360	6, 970
DL-Phenylalanylglycine (L)	300	900

[1] Digests and experimental conditions as in table 4.

SUMMARY

The activity of certain enzyme systems which act upon closely related substrates has been studied in rat liver and primary hepatoma and in mouse liver and transplanted hepatomas 98/15 and 112B.

The activity of glutaminase I in liver increases on cancerization, that of glutaminase II diminishes. The activity of these enzymes in fetal liver follows the hepatoma pattern.

Asparaginase II activity of liver diminishes on cancerization.

The activity of certain members of the dehydropeptidase I group in liver increases on cancerization; the others, together with dehydropeptidase II, diminish or disappear.

The activity of the enzyme active on N-acylated amino acids in liver is virtually unchanged when the liver becomes neoplastic. With substrates containing a free α-amino group adjacent to the peptide bond, the activity in the mouse hepatoma 112B becomes concentrated, that in the rat hepatoma may increase or decrease depending upon the configuration of the substrate.

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CARDIOVASCULAR EFFECTS PRODUCED BY STILBAMIDINE IN PATIENTS WITH MULTIPLE MYELOMA [1] [2]

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The stimulating report by Snapper (*1*) on the therapeutic effectiveness of stilbamidine (4,4′-diamidinostilbene) in patients with multiple myeloma has resulted in renewed interest and more extensive investigation of the drug.

In the extensive review of the aromatic diamidines by Schoenbach and Greenspan (*2*) the effects of stilbamidine on the cardiovascular system are summarized by the statement that, "despite the frequently marked transient vasodepression following intravenous injections of the diamidines in man, no evidence of cardiac toxicity per se has been reported."

The diamidines have been noted to have a marked depressive action on the blood pressure, allegedly attributable to direct vasodilation in the smaller arteries and arterioles (*2*). Wien (*3*) found in isolated heart-lung preparations, that 1:2,000 stilbamidine produced an increase in heart rate; with higher concentrations it had a depressing action. The minimal and inconstant changes suggested no direct effect upon the heart.

In the course of clinical studies with stilbamidine in the treatment of multiple myeloma, it was noted that severe cardiovascular symptoms often occurred. These symptoms have been previously noted (*1, 4*). This study was initiated in view of the paucity of data regarding the effects of the drug on the electrocardiogram and pulse contour.

SUBJECTS AND METHODS

Four patients with multiple myeloma proven by biopsy were given a total of 28 intravenous injections of stilbamidine.[3] They were studied by clinical observation, continuous recording of multiple simultaneous precordial electrocardiograms, pulse, and respiration tracings (*5*),[4] and oxygen arterial saturation by continuous oximetric determinations. Varying rates of administration were employed to determine the relationship of speed of administration to the cardiac symptoms and signs. One hundred fifty mg. of the material was diluted in 4 cc. of sterile distilled

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[2] Aided by Grant 396 from the National Advisory Cancer Council.
[3] Kindly supplied by Merck & Co., Rahway, N. J.
[4] BIERMAN, H. R., RAPAPORT, E., and JENKINS, R. J.: Multiple electrocardiography. Unpublished data.

TABLE 1

Patient	Date of injection	Stilbamidine	Duration of injection	Time of onset		Fall in O_2 saturation	Time of onset of recovery	Symptoms	Maximum change (in seconds) and comment
				T-wave changes	Pulse contour				
		Mg.	*Seconds*	*Seconds*	*Seconds*	*Percent*	*Seconds*		
L. P.	June 23 (1)	50	120	90			220	Hyperpnea; warmth, flushing, paresthesia, sweating, hyperpnea.	175; T broad and flat V_5, V_6. 90; rate 120 ST depr. V_{4-6}.
	(2)	50	19	45			120		
	June 25	150	120	No change				None	No change.
	August 25	150	30	12			90	Paresthesias, hyperpnea, flushing.	70; ST depressed V_3 V_4, V_6 diphasic T_4, T_6; rate 108.
	September 2	150	10	50			180–240	Subst. oppress; paresthesia; hyperpnea; slight cyanosis; sweating.	75; rate 125; ST depressed 3 mm. V_3, V_4 V_6; diphasic T V_4, V_6.
S. T.	July 7	75	60	30			175	Tightness of chest; paresthesia; hyperpnea.	105; rate 140 ST depressed 2 mm. in V_{4-6}. P fused with T waves.
	July 9	150	60	60		None	150	Hyperpnea, dizziness	75; rate 114 ST depressed T low in V_{4-6}.
S. G.	December 23	75	240	28	28	91 to 82	75	Hyperpnea; twitches; paresthesia; cyanosis.	28; T slightly flatter, fall in dicrotic notch.
	December 24	100	30	64	64	91–79	130	Paresthesias, twitches, sweating, cyanosis.	95; slightly depressed ST V_6; T lower V_4–V_6; dicrotic notch on anacrotic limb.
	December 26	150	60	50	50		110	Same but slightly less severe than December 24.	85; rate 124 ST depressed V_4–V_6; dicrotic notch at baseline.
	January 2	150 [1]	240	85	35	93 in 6	180	Dyspnea, slight	85; slight ST depressed V_4–V_6. (minimal).
	January 9	150	180	90	90	93 to 86	240–360	None	Slight ST depression without change in pulse rate; fall dicrotic notch to baseline.
	January 12	150	160			93 to 86	180	Hyperpnea	
	January 14	150	60	50			240	Dyspnea, nausea; erythema	180; rate 156; ST depressed 3 mm. in V_4; Fall of dicrotic notch to baseline; T flat in V_4–V_6.
	January 16	150	180	No significant change.	No significant change.	93 to 91	No significant change.	None	No significant change.
	January 19	150	240	No significant change.		None	No significant change.	None	No significant change.
	January 30	Atropine 1/150.				None	No significant change.	None	Rate 138. Minimal ST depression. P and T waves fused. No T change.
E. P.	April 8 (1)	50	120	No change	None	95 to 90	No significant change.	Slight sweating and paresthesia	No significant change.
	(2)	50	60	180	180	95 to 90	300	Slight dyspnea	Slight ST depression V_4–V_6; fall in dicrotic notch.

[1] PBZ 1 cc. (25 mg.) 20 minutes before stilbamidine.

water. The injections were administered intravenously without further dilution. The pH of this solution was 5.6 ± 0.2 and was not considered sufficiently acid to be of significance.

RESULTS

The cardiovascular effects of 28 separate intravenous injections of 50 to 150 mg. of 4-4′-diamidinostilbene (stilbamidine) in 4 patients with multiple myeloma are summarized (table 1). In all instances there was some degree of dermal erythema, increase in the rate and force of the heart beat, substernal oppression, increase in the rate and depth of respirations, diaphoresis, and paresthesias. In general, the severity of the symptoms and signs was directly related to the rapidity of injection. The effects of the initial injection were usually the most striking despite the use of such small doses as 75 mg. in comparison to subsequent injections of 150 mg.

In most instances the findings were proportional to the severity of the cardiovascular symptoms. The predominant changes were:

1. Electrocardiographic:
   (a) Depression of the ST segments, especially in V_4 to V_6.
   (b) Lowering of the T wave especially in V_4 to V_6.
   (c) Sinus tachycardia.
2. Pulse tracings:
   (a) Fall in blood pressure.
   (b) Fall of the dicrotic notch.
   (c) Change in the contour of the catacrotic limb of the pulse.
3. Oxygen saturation:
   (a) Prompt fall to as much as 9 percent.

The electrocardiographic or pulse contour changes occurred within 2 minutes of the onset of the injection, persisted for 3 to 4 minutes, and gradually subsided after 5 to 7 minutes (figs. 1, 2, 3). The changes in the pulse contour usually were the first significant abnormaliity noted, although in some instances they were preceded by the electrocardaographic abnormalities. The changes had completely disappeared in all cases within 15 minutes. In no case were there permanent electrocardiographic or pulse wave changes.

The abnormalities noted were most marked when 150 mg. of the drug was given within 1 minute (figs. 1, 2) and were correspondingly less severe when the injection time was lengthened to 3 minutes (fig. 3). Fall in both systolic and diastolic blood pressure occurred at 50–130 seconds following the start of rapid injection (figs. 1, 2). With slower injection, the fall in blood pressure was minimal or absent (fig. 3).

DISCUSSION

Immediate significant changes in pulse contour and the electrocardiogram occurred following the intravenous administration of stilbamidine to four patients with multiple myeloma. The cardiovascular effects appeared to be related to the speed with which the drug was injected, because the severity of the symptoms and the cardiovascular changes were

[1] PBZ 1 cc. (25 mg.) 20 minutes before stilbamidine.

less marked when the drug was given over at least a 3-minute period as compared to more rapid injection. In some instances, when the drug was administered slowly, no significant cardiovascular symptoms or signs occurred. The findings are suggestive of both myocardial and peripheral effect.

Hypotension may be a causative factor because of the fall in both systolic and diastolic blood pressure noted at the time of appearance of the pulse contour and electrocardiographic changes (fig. 2, C). However, the pulse contour and electrocardiographic abnormalities were still present when the systolic blood pressure had returned to the control level (fig. 2, E). With slower rates of administration, the paucity of symptoms were in accord with the absence of blood pressure change and the minimal to moderate pulse contour abnormality (fig. 3).

The fall in the dicrotic notch suggests a lowering of the mean arterial blood pressure; this in turn could result in decreased coronary blood flow. The fall in oxygen saturation and the substernal oppression could be related to coronary insufficiency. The role of tachycardia may be significant because of the rapid cardiac rate resulting from intravenous stilbamidine. Observations following atropine alone revealed similar electrocardiographic changes, but at times the electrocardiographic and pulse contour abnormalities were noted without important tachycardia.

Varying degrees of auriculoventricular block including complete auriculoventricular dissociation have been noted when stilbamidine was administered to mice,[5] but conduction defects were not observed in any of the cases in this series.

The cardiovascular effects obtained with stilbamidine were transient, and in every case the pulse contour and electrocardiogram returned to their preinjection contour within 15 minutes.

The above observations suggest that it is desirable to administer stilbamidine slowly if the drug is used intravenously, particularly in the older age groups.

SUMMARY AND CONCLUSIONS

Rapid intravenous administrations of stilbamidine to four patients with multiple myeloma were followed by:

(a) Symptoms of substernal oppression, pounding of the heart, paresthesias of the face and body, and sweating.
(b) Fall in blood pressure.
(c) Depression of the ST segment and lowering of the T wave, preponderantly in the left precordial leads.
(d) Sinus tachycardia.
(e) Fall in the dicrotic notch and change in the contour of the catacrotic limb of the arterial pulse tracing.
(f) Fall in arterial oxygen saturation to as much as 9 percent.

[5] BIERMAN, H. R., RANDOLPH, E., SOKOLOW, M.: Effect of topic doses of 4,4'—diamidinostilbene (stilbamidine) on the electrocardiogram in mice (in preparation).

The immediate cardiovascular effect of stilbamidine when given intravenously was greater when the drug was administered rapidly (within 1 minute) than if given over a period of 3 or more minutes. The cardiovascular effects may be minimal or absent when the slower rate of administration was employed.

The changes are suggestive of a combined peripheral and myocardial effect of stilbamidine. Hypotension and tachycardia probably play a significant role.

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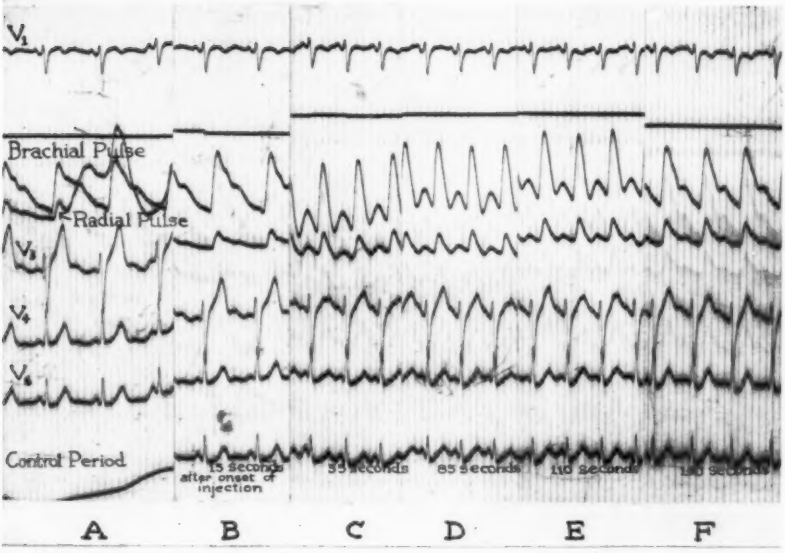
(*4*) Stilbamidine in the treatment of multiple myeloma, Medical Division, Merck & Co., Inc., Rahway, N. J. (1948).

(*5*) BIERMAN, H. R.: Device for measuring physiological pressure phenomena using the bonded electrical wire resistance strain gage. Rev. Scient. Instruments 19: 707–710 (1948).

PLATE 53

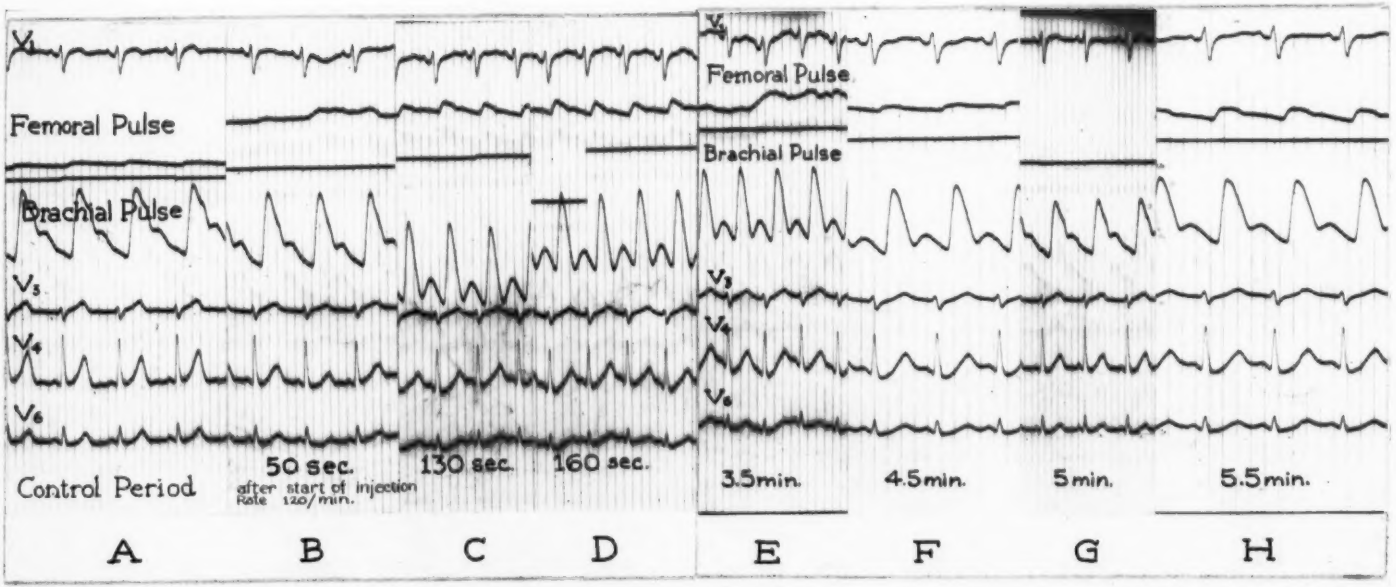
FIGURE 1.—Acute onset of electrocardiographic, pulse contour, and blood pressure changes following rapid injection of 150 mg. of stilbamidine. All data recorded simultaneously.



S.G. ♂ AGE 53 12-26-47
150 Mg. STILBAMIDINE
INJECTION PERIOD 60 SECONDS

PLATE 54

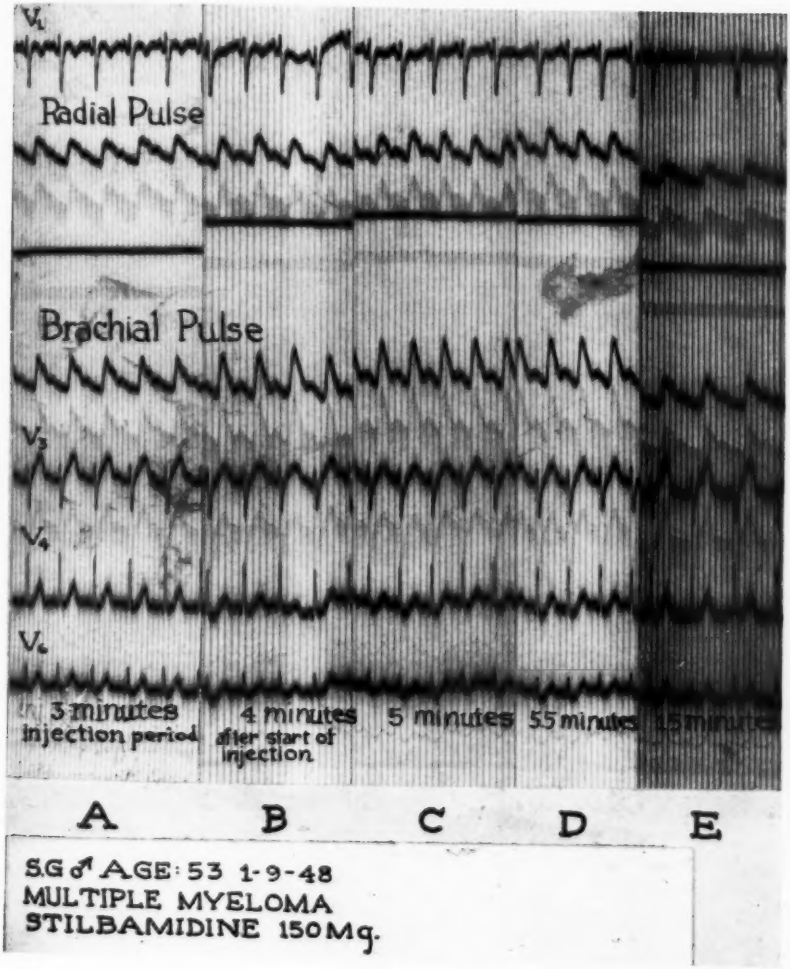
FIGURE 2.—Rapid injection of 150 mg. of stilbamidine. Note change in pulse contour, fall in blood pressure and ST, T changes in C, D, and E. All data recorded simultaneously.



S.G. ♂ AGE: 53 1-14-47
MULTIPLE MYELOMA
STILBAMIDINE 150 Mg. I.V.
INJECTION PERIOD 60 SECONDS

PLATE 55

FIGURE 3.—Slight changes following slow administration of 150 mg. of stilbamidine. All data recorded simultaneously.


V1
Radial Pulse
Brachial Pulse
V3
V4
V6
3 minutes injection period
4 minutes after start of injection
5 minutes
55 minutes
A
B
C
D
E
S.G ♂ AGE: 53 1-9-48
MULTIPLE MYELOMA
STILBAMIDINE 150 Mg.

INFLUENCE OF PERFORATION DESIGN ON GROWTH OF TISSUE CELLS UNDER PERFORATED CELLOPHANE SHEETS IN VITRO [1]

By WILTON R. EARLE, VIRGINIA J. EVANS, MARY-FRANCES EDWARD, and EMILY DUCHESNE, *National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.*

INTRODUCTION

Evans and Earle (*1*) have shown that perforated cellophane can be used as a substrate for the growth of a number of cell strains in Carrel flasks. The data in the previous study indicated that strain 224 mouse fibroblasts had difficulty in adhering to the smooth surface of the cellophane but were able to adhere to the rough edges of the perforations. Strain L mouse sarcoma cells grew luxuriantly and had no difficulty in adhering. In the present study, using these two cell strains, an attempt has been made (1) to determine the influence of the size and pattern of cellophane perforation on the growth of the cultures, (2) to determine which sizes and patterns of cellophane perforations are best suited for routine work with these cell strains, and (3) to present certain additional data pertinent to use of this substrate in tissue culture.

MATERIALS AND METHODS

Modified Carrel D flasks 3.5 cm. in diameter were used as culture vessels. All cellophane used was DuPont plain transparent PT-62, thickness 450. This was perforated by the Beckley Perforating Co., Garwood, N. J. As described in the catalog of this company, the design used by Evans and Earle (*1*) and the four designs used in the present study were as follows:

(1) *Design 3B.* Used by Evans and Earle in previous study. Holes .040 inch diameter; 45° centers; 112 holes per square inch; 14 percent open area.

(2) *Design 00.* Holes .020 inch diameter; regular staggered pattern; 625 holes per square inch; 20 percent open area.

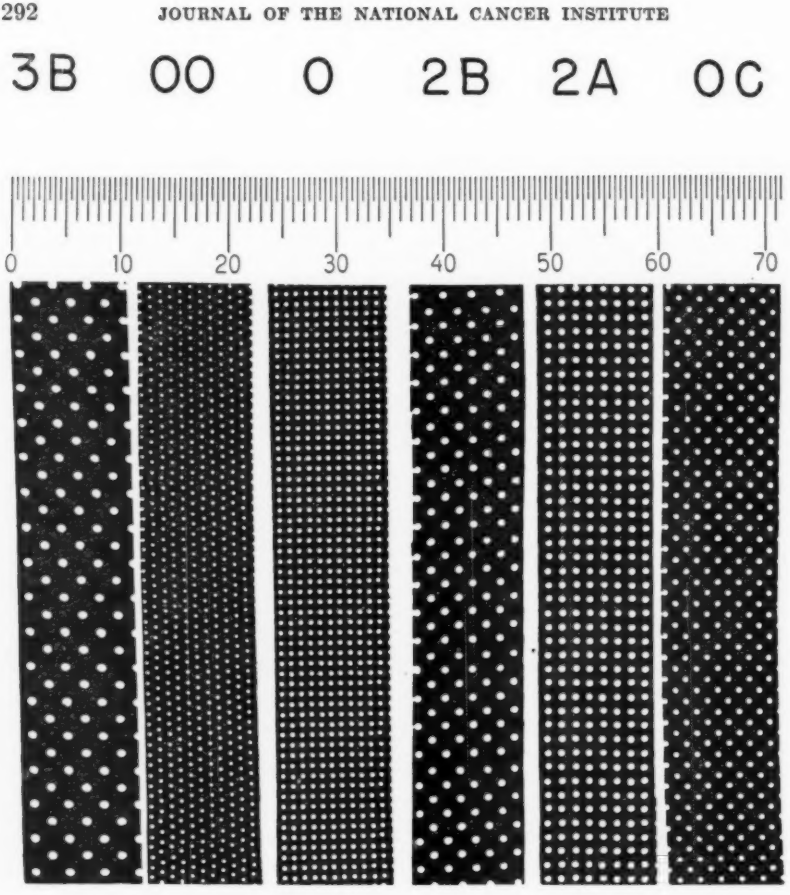
(3) *Design 0.* Holes .023 inch diameter; straight line centers; 576 holes per square inch; 22½ percent open area.

(4) *Design 2B.* Holes .033 inch diameter; 45° centered holes; 162 holes per square inch; 14 percent open area.

(5) *Design 2A.* Holes .033 inch diameter; straight line centers; 324 holes per square inch; 28 percent open area.

These designs are shown in text-figure 1. There were minor deviations in some of these designs apparently due to some shrinkage in the cellophane.

[1] Received for publication August 16, 1949.



TEXT-FIGURE 1.—Enlarged contact print of actual strips of cellophane to show different perforation designs. The identification of the first five designs corresponds to that in the Beckley catalog; Design OC is described on page 294 in the present manuscript. The scale is graduated in millimeters.

The cellophane was cut into circles to fit the bottom of the flasks. In the cutting of these circles, allowance was made for shrinkage in autoclaving. The circles of cellophane were washed in two changes, 15 minutes each of acetone, ether, alcohol, and triple-distilled water. The cellophane was then dried and inserted in the flasks. The flasks were sterilized by autoclaving. In all cellophane cultures 1 ml. of fluid culture medium, which consisted of 40 percent horse serum, 20 percent chick embryo extract (1:1) and 40 percent saline (*2*), was added to each flask. Each cell explant was inserted into a culture flask and centered, by means of a platinum spatula (*2*) beneath the cellophane sheet, which had been soaked for 1 minute with the fluid culture medium. The flasks were sealed and incubated at 37° C.

Control cultures in plasma clot matrix were included with each series. In these the plasma clot consisted of 0.6 ml. chicken plasma and 0.8 ml. of the fluid culture medium. As a supernatant fluid in the plasma cultures 1 ml. of the above fluid culture medium was used. Three times weekly the fluid culture medium was withdrawn from cultures and replaced with fresh.

Photographs of representative cultures were taken with the camera previously described (*3*) at 21 to 23 days after the date of planting. These photographs were used for comparison of the characteristics of the cultures.

Two strains of cells were used in this study. The first was a strain of subcutaneous mouse fibroblasts carried in vitro, since January 7, 1944, in this same plasma, horse serum, chick embryo medium. This strain designated as strain 224 (*1*), was of presumably normal cells but on recent injections has given rise to some sarcomas at the site of injections. The second strain used was strain L mouse sarcoma, the behavior of which has already been described (*1*, *2*).

RESULTS

The plasma controls of both strain 224 and strain L at 6 to 12 days after planting showed moderate fringes of cells extending about half-way from the original explant to the edge of the flask on all sides, while at 24 days a majority had cell sheets to the edges of the flask.

Strain 224 cells under unperforated cellophane grew only slightly, if at all. But the strain L cultures showed rapid and luxuriant growth comparable in extent to that shown by strain L under the perforated cellophane.

Under designs 00 and 0, cellophane growth of strain 224 cells at 6 days after planting was slight. There was only a limited migration of cells from the twelfth to the twenty-fourth day. Cell foci were concentrated around the roughened edges of the perforations. Cultures under designs 2B and 2A cellophane migrated at possibly an even slower rate than on designs 00 and 0. Growth of this strain under any of the cellophane sheets, however, was possibly slightly inferior to that in plasma.

Growth of strain L cultures under all four types of perforated cellophane was rapid and extensive. Isolated cell clusters soon appeared over the entire surface of the cellophane. Later in most cases, cell growth was so great that the cells were heaped up several layers thick over the whole flask-floor area. At 21 to 23 days, necrosis had begun to appear in some cultures, probably due to the crowding of the densely packed cells.

DISCUSSION

From these results on two cell types of such dissimilar behavior under cellophane, it appears that within the range of patterns tested, the perforation design of the cellophane is a minor factor in the satisfactory use of cellophane in the culture. Strain L grew well under all designs of perforated cellophane and equally as well under unperforated cellophane.

Strain 224 showed only a limited difference in its behavior under any of the four designs of perforated cellophane studied; its cultures did appear to do better under cellophane having closely spaced, finer perforations, such as in the 00 or 0 designs. Such closely spaced holes as in these designs were found, however, to interfere unduly with both visual and photographic records. This fine a perforation was also mechanically difficult to obtain in large quantities.

On the basis of these observations, a perforation design was selected for further routine use in this laboratory. The specifications for this perforated cellophane are: DuPont Cellophane [2] PT 62, thickness 450, with Beckley Perforation design OC; hole diameter .023 inch, diagonal pattern 45° centers, 324 holes per square inch, 13½ percent open area. This perforation design OC had small perforations, the size of those in design 0. The perforations were spaced somewhat farther apart however, so as not to interfere unduly with photographic and visual records of the cultures. This type perforated cellophane has now been used for the growth of more than 10 cell strains in this laboratory during the last year and has been found satisfactory.[3]

With respect to the cleaning of the cellophane used in culture work, the procedure being routinely used for further work in this laboratory calls for 3–10-minute changes each of acetone, ether, alcohol, and distilled water instead of the two 15-minute changes of solvent reported in this study.

In later work it has been found that when cellophane was repeatedly autoclaved it turned brown and became brittle. Cellophane not used after one autoclaving is, therefore, routinely discarded.

It was not realized until this study was practically complete that the two sides of the perforated cellophane sheets are not identical. As the die perforating the cellophane enters the sheet, the cellophane is pushed out by each boss on the die. The result is that the side of the sheet from which the die emerges is slightly elevated around each perforation and is slightly rough to the touch. In further work where a precise control of culture conditions is desired, it may be advisable to use the cellophane with its rough surface uniformly toward the glass. From what has been observed, this factor is considered an extremely minor one in the growth of the culture.

One difference in the general behavior of cultures in plasma and under cellophane deserves emphasis. In plasma the cells migrate out from the explant as a fringe and gradually spread in an approximately even pattern to the edges of the flask. Cultures under cellophane appear to begin their increase in area by sending out a similar fringe of cells, but soon isolated cells and cell clumps break loose, are widely distributed over the surface, and there continue to grow. In this respect the manner of increase in the

[2] Personal communication from the DuPont Co. states that as used in this study (following washing to remove such softeners as glycerine) this type cellulose film may be considered as pure regenerated cellulose.

[3] Such perforated cellophane is now available from the Tissue Culture Commission Laboratories, Sloan-Kettering Institute, 444 East 68th St., New York City 21.

area of cells in a cellophane culture is similar to that usually seen in rapidly proliferating plasma cultures which show liquefaction of the plasma matrix. In these cultures which show liquefaction of the plasma, there is frequently the liberation of loose cells and cell clumps which reanchor on the surface of other areas of the plasma and there continue proliferation.

SUMMARY

The results of this study of the influence of perforation design on the growth of cells of strain L mouse sarcoma and strain 224 cells under perforated cellophane sheets indicate that perforation design is of minor significance.

On the basis of data available, a standard type of perforated cellophane sheet for routine tissue culture work is suggested.

Additional technical details on the use of cellophane in tissue culture are given.

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(*1*) EVANS, V. J., and EARLE, W. R.: The use of perforated cellophane for the growth of cells in tissue culture. J. Nat. Cancer Inst. 8: 103–119 (1947).

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PROCEEDINGS OF THE FOURTH CONFERENCE ON GASTRIC CANCER[1]

The Fourth Conference on Gastric Cancer sponsored by the Gastric Cancer Committee of the National Advisory Cancer Council, National Cancer Institute, National Institutes of Health, United States Public Health Service, was held in Lane Hall, Stanford University School of Medicine, San Francisco, California, December 13 and 14, 1948.

GASTRIC CANCER COMMITTEE
OF THE
NATIONAL ADVISORY CANCER COUNCIL
TO THE
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH,
UNITED STATES PUBLIC HEALTH SERVICE

Committee Members

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Executive Secretary: Morris K. Barrett, M. D., *National Cancer Institute, Bethesda, Md.*

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HENRY S. KAPLAN, M. D., *Stanford University, San Francisco (appointed July 1949)*
SHERWOOD MOORE, *Washington University, St. Louis (term expired July 1949)*
CLARENCE P. OLIVER, Ph. D., *University of Texas, Austin (appointed July 1949)*
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JOHN P. QUIGLEY, Ph. D., *University of Tennessee, Memphis (appointed July 1949)*
GEORGE M. SMITH, M. D., *Yale University, New Haven*
ARTHUR PURDY STOUT, M. D., *Columbia University, New York (appointed July, 1949)*
LEONELL C. STRONG, Ph. D., *Yale University, New Haven*
SHIELDS WARREN, M. D., *New England Deaconess Hospital, Boston (term expired July 1949)*

[1] The Journal of the National Cancer Institute is serving as the organ of publication for the Fourth Conference on Gastric Cancer as it has previously done for the preceding conferences.

SCIENTIFIC PROCEEDINGS

MONDAY MORNING SESSION
December 13, 1948

Address of welcome.....JOHN R. HELLER
The use of fluorescein dyes in the diagnosis of malignancy with special reference to tumors of the central nervous system.....T. B. HUBBARD and G. E. MOORE
The metabolism of the stomach.....HORACE W. DAVENPORT
Studies in histochemistry. XX. Urease in the human stomach with respect to acid secretion in ulcer and cancer.....DAVID GLICK
Effect of intravenous glucose on gastric lactic acid in gastric carcinoma
BERNARD SHACTER, RALPH L. BYRON, Jr., and MICHAEL B. SHIMKIN
The inhibition of gastric secretion in dogs by human gastric juice and gastric mucin
CHARLES M. BLACKBURN and CHARLES F. CODE
Histological study of the destruction and regeneration of the gastric mucous barrier following application of eugenol, preliminary report
FRANKLIN HOLLANDER and RHODA L. GOLDFISCHER
Preparation of emulsions and suspensions containing carcinogenic hydrocarbons
EGON LORENZ
Adenocarcinoma and other lesions of the glandular stomach of mice following intramural injection of 20-methylcholanthrene
HAROLD L. STEWART, W. V. HARE, E. LORENZ, and J. G. BENNETT
The occurrence of gastric lesions in the rat as a result of feeding tributyrin
W. D. SALMON and D. H. COPELAND
Gastric cancer experimentally induced in insects by nerve severance..BERTA SCHARRER
The role of mucous cells in the production of gastric neoplasms..EDWARD L. HOWES

The Fourth Conference on Gastric Cancer was convened at 9:00 a. m., Dr. Owen H. Wangensteen of the University of Minnesota presiding.

Chairman Wangensteen: Ladies and Gentlemen, let us come to order. We have a rather long program, and we do want to get it all in.

As I recall, the New Testament says that it is more difficult for a rich man to get into Heaven than for a camel to get through a needle's eye, but after getting in here this morning, I believe it is more difficult to get into the Gastric Cancer Conference.

This is the fourth meeting of the general cancer biologists constituted by a wide group. It is good, I think, for us to get together periodically to fuse our knowledge, even though no startling information may come out of these presentations. If any of you has been surprised this morning at hearing no preliminary announcements, I remind you that an announcement of very startling news in San Francisco may be a somewhat hazardous thing; so no world-shaking announcements will be made, I hope, without giving us some warning thereof.

We have the greatest pleasure of having with us this morning Dr. J. R. Heller, who is Director of the National Cancer Institute. He is here on behalf of the United States Public Health Service and the Cancer Council, under the auspices of which we are meeting. At this time we will open the sessions with a message from Dr. Heller.

Dr. Heller: Dr. Wangensteen, ladies, and gentlemen, I should like to read a telegram from Dr. Scheele:

PLEASE TELL MEMBERS GASTRIC CANCER CONFERENCE I DEEPLY REGRET URGENT MATTERS PUBLIC HEALTH SERVICE BUSINESS HAVE ARISEN TO PREVENT ATTENDANCE MEETING SAN FRANCISCO. GREAT DISAPPOINTMENT TO ME BECAUSE I EAGERLY LOOKED FORWARD ATTENDING CONFERENCE. LEONARD SCHEELE

Dr. Scheele requested that I additionally express his regret at not being able to join the group. It is a keen disappointment to him, but he wants you to know that he is immensely interested and hopeful that much of great value will come from the conference and that it will stimulate additional work on gastric cancer throughout the Nation.

ADDRESS OF WELCOME [1]

By JOHN R. HELLER, *Director, National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.*

It is a pleasure to welcome you to the Fourth Conference on Gastric Cancer sponsored by the National Advisory Cancer Council.

No conference in the medical field could be more timely or of more vital concern to the public and the medical profession. Cancer of the stomach, as compared with other diseases and other types of cancer, is one of the most difficult to discover and diagnose, and for that reason one of the most difficult to control.

All of us in the field of cancer control—from the general practitioner to the cancer specialist to the public health officer—look to the men of science who are gathered at this conference to forge better tools to help us deal with the stomach cancer problem. We are most eager to put them into effect and thereby reduce the shocking annual toll of at least 25,000 deaths due to gastric cancer. As the first-ranking cause of cancer deaths among males, gastric cancer must be attacked as a major enemy.

Our problem is not that we cannot cure early cases of gastric cancer; surgical techniques and the control of secondary infections have scored amazing advances. Time was when the radical surgery usually indicated for stomach cancer was tantamount to a death warrant; now it is relatively safe, and a gratifying proportion of patients successfully come through the operation itself. The difficulty is rather that in about half his gastric cancer cases the surgeon—as well as the patient—is denied a fair chance. By the time the case is diagnosed, the chances are approximately even that the disease is too far advanced for a curative operation. Even at that advanced stage, of course, surgery can often do much to increase the patient's comfort and prolong his life.

Our greatest need at this time is to find gastric cancer early—to detect it, diagnose it, and bring it to the surgeon's table while it is still curable. Although we have no cause for pessimism, there is no point in minimizing the difficulty of this problem. For early gastric cancer is silent and usually presents no symptoms that definitely indicate cancer. Often the only early symptom is a vague and persistent stomach discomfort, which can too easily be ascribed to a minor upset that seems to require only simple home remedies. But this minor symptom, which may or may not be accompanied by indigestion, heartburn, gas, nausea, unexplained weight loss, or loss of appetite, is often the only warning that occurs. Usually it does not mean gastric cancer, but often it does, and it is, therefore, dangerous to ignore it.

[1] Presented at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

Workers in the control field, both in the voluntary and the public agencies, are striving to make the public conscious of the importance of those symptoms. I venture to think that our campaigns are paying dividends and that the proportion of early discovered cases is on the increase. But no one would deny that we need new and better detection tools.

We know that progress is being made. New techniques are being applied in histochemistry, physiology, and exfoliative cytology; gastric cancer surveys have been undertaken both by diagnosticians and by geneticists; improved methods of photoflûorography have been developed; there are new leads concerning enzymes in the gastric mucosa and gastric secretions; and studies of environmental and dietary factors are under way.

For a great part of this progress the public and the medical profession owe a major debt to the men and women who devoted their energy and imagination to making the first three Conferences on Gastric Cancer a success and to the National Advisory Cancer Council which sponsored and directed them.

The story of the gastric cancer conferences, as most of you know, goes back to 1939, when the National Advisory Cancer Council appointed a committee to consider the subject of cooperation in cancer research. After a survey of the types of cancer studies that were receiving financial support from various sources at that time, the committee decided that one of the most neglected phases of the problem was gastric cancer. It seemed imperative that something be done, if for no other reason than to change the pessimistic attitude which prevented research workers from attacking the problem. It was thought that by bringing together a group of research workers for discussion of the problem, interest would be stimulated and ideas for ways of attacking the problem would result. This proved to be a correct assumption.

The first gastric cancer conference was held at the National Institutes of Health, Bethesda, Md., in October 1940. It clarified the gastric cancer problem, suggested important lines of investigation, and dispelled to a considerable degree the previous pessimism surrounding the problem. Following this conference, greater interest in gastric cancer was manifested by research workers, and this has increased as time has gone on.

Two people more than any others deserve our thanks for originating these conferences and keeping them going: Dr. James B. Murphy and Dr. George M. Smith. It was Dr. Murphy who urged the wisdom of concentrating on the difficult problem of gastric cancer instead of confining our efforts to simpler problems which might be easier of solution. Dr. Smith, as chairman of the Council's gastric cancer committee, put all his energies into developing the program once it had been started.

In order that the gains made by the first gastric cancer conference might not be lost in the pressing problems of the war, a second but smaller conference was held in 1944 at Memorial Hospital in New York City. As soon as practical after the close of the war, a third and larger

conference was held at the University of Chicago in December 1946.

Many of you have attended at least one of these conferences, and some of you have been present at all of them. You know better than I—this is the first I have attended—how much of a factor they have been in stimulating the expanded gastric cancer research program that is being carried on today. This year the National Cancer Institute alone is supporting 20 gastric cancer projects in 14 institutions by grants-in-aid totalling $181,700, and these are by no means the major part of the research work now going on. All of us are eager for a progress report on this work—much of which will be described at the sessions to follow.

We can open this conference today in a spirit of optimism with full confidence that it will contribute to further advances in our understanding of the gastric cancer problem.

Chairman Wangensteen: Thanks, Dr. Heller, for this encouraging message. Before we proceed with the program I should call on Dr. Northway, Leland Stanford Medical School, Lane Hospital, to say something to us concerning the opportunity we are enjoying in meeting here.

I should like to preface my remarks in speaking of the difficulty which confronted our committee in deciding where to meet here in San Francisco, by saying that I come from Minneapolis and St. Paul—I do come from Minneapolis—I can't live in both municipalities. Just recently Dr. Ravdin, in Philadelphia, having in mind a visit he made me, sent me a clipping from the papers indicating that a well-known St. Paul lawyer, Mitchell by name, had gone to Philadelphia to talk. He had talked about the difficulty they were having, and he said, "It it nothing compared to the difficulty we are having in St. Paul and Minneapolis. They took up a collection in St. Paul the other day, and just before the conclusion of the church service the deacon, said, 'We took in $60.03. There must be a visitor from Minneapolis.' Thereupon someone got up in the back and said, 'We have three visitors from Minneapolis.' " (Laughter.) Dr. Northway—

Dr. Northway: On behalf of Dean Chandler, who was unable to come here this morning, I want to welcome you and the Fourth Gastric Cancer Conference to the City of San Francisco and to Leland Stanford University.

We haven't any pretentious hall, as you can see, to welcome you to, but we hope you have a good time while you are here. We hope that you learn a good many things, and we want you to feel at home while you are here. Welcome to you! (Applause.)

Chairman Wangensteen: Thank you, Dr. Northway. The first paper on the program is entitled The Use of Fluorescein Dyes in the Diagnosis of Malignancy with Special Reference to Tumors of the Central Nervous System. I suppose it is bad to start a program with an apology, but the paper is to be presented by Dr. George Moore from the University of Minnesota, and I am hopeful that Dr. Moore will be able to connect his paper in some way with our program on gastric cancer. Dr. Moore—

THE USE OF FLUORESCEIN DYES IN THE DIAGNOSIS OF MALIGNANCY WITH SPECIAL REFERENCE TO TUMORS OF THE CENTRAL NERVOUS SYSTEM [1] [2]

By T. B. Hubbard, M. D., and G. E. Moore[3], M. D.,
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The relative localization of dyes in neoplastic tissues has been described by numerous investigators in the past (*1–8*). In this clinic the chance observation was made that peritoneal tumor implants fluoresced when viewed under ultraviolet light in a patient who had previously received an intravenous injection of fluorescein. Therefore, a study was begun of the localization of this dye in tumors, particularly of the gastrointestinal tract and of the brain (*9*).

GASTROINTESTINAL LESIONS

Procedure

The dye was injected intravenously from 3 to 11 hours before the abdomen was examined. Dosage varied from 2.5 to 10 cc. of 20-percent sodium fluorescein. In 10 cases, multiple dosages at 24-hour intervals were given for 2 to 4 days before operation, with no difference in results. Examination was carried out under the illumination of a CH–4 mercury vapor lamp with a Wood's filter emitting rays at about 3,600°. Most tumors were examined in situ and also after excision. In the latter case, both serosal, mucosal, and cut surfaces of the tumor and normal bowel were compared.

Results

(See table 1.) Thirty-six cases of carcinoma of the stomach were examined. Of these, 17 were considered excellent or good results, and 19 were graded as poor or failures. Seven cases were of a scirrhous type, 21 were adenocarcinomas, and 4 were considered as poorly differentiated carcinomas. Four unresectable cases were based only on the gross diagnosis. There was no significant difference in results obtained with the various histological types. Two cases of squamous-cell carcinoma of the distal third of the esophagus were studied, with 1 good and 1 poor result. One case of lymphosarcoma of the stomach showed no fluorescence.

Fifteen cases of carcinoma of the colon were examined. Of these, only 2 were graded as good, and 13 were graded as poor.

[1] Supported in part by grants from the National Cancer Institute, the Flora L. Rosenblatt Fund for Cancer Research, and the Malignant Disease Research Fund.

[2] Read by G. E. Moore at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

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TABLE 1.—*Summary of results after fluorescein injection*

Diagnosis	Cell type	Number of cases	Results				
			Excellent	Good	Poor	Failure	Indeterminent
Carcinoma of the stomach	Scirrhous	7	1	3	2	1	
	Adenocarcinoma	21	1	6	10	4	
	Poorly differentiated.	4	0	2	2	0	
	Gross carcinoma	4	1	3	0	0	
Total		36	3	14	14	5	
Lymphosarcoma of the stomach.		1			1		
Squamous-cell carcinoma of the distal esophagus.		2		1	1		
Carcinoma of the colon		15		2	13		
Central nervous system tumors.		91	87			3	1

Eleven cases of benign duodenal or gastric ulcers were examined. In the case of 3 chronic duodenal ulcers, the scarred serosa over the ulcer fluoresced quite markedly. This was also the case with one of the gastric ulcers, which was characterized by considerable scar tissue penetrating the depth of the stomach wall.

Numerous miscellaneous lesions of the bowel were examined, the most significant of which were a perforated diverticulum of the colon and a benign endometrioma of the colon, both of which fluoresced well.

The results described above must be qualified somewhat. In many cases of carcinoma, graded as good or excellent, the fluorescence was relative, in that the peritoneal fat, especially in the immediate vicinity of tumor, would also fluoresce. For example, in one case of carcinoma of the stomach, the tumor was well outlined by fluorescence, but when separated from the transverse mesocolon to which it was adhered, the mesocolon in that area fluoresced equally well. Sections of this tissue showed only lymphoid follicles and loose connective tissue. In a metastatic adenocarcinoma of the cecum, certain parts of the tumor nodules in the mesentery fluoresced well, but a considerable amount of the grossly uninvolved mesentery in that area fluoresced equally well. Biopsy of the latter revealed only fat with some chronic inflammatory infiltration about the blood vessels in the section. Both of these cases have been graded as good.

The dye has not been visualized microscopically directly in relation to the involved tissues. However, as in the above cases, sections have been taken in a number of cases in appropriate fluorescent and nonfluorescent areas. In situ a tumor never fluoresced unless it was subserosal or on the surface, since ultraviolet rays are stopped by even a thin layer of tissue. Thus, peritoneal implants presented some of the most satisfactory results. On cut section of the excised specimens, the fluorescence, when present,

was usually relegated to the periphery of the tumor and/or to patchy areas throughout the mass. Microscopic examination of the periphery of such tumors revealed, in the approximate area of fluorescence, the usual false capsule composed of fibrous connective tissue with varying amounts of chronic inflammatory infiltration and edema. In the one case where the patchy areas of fluorescence deep in a tumor mass were of sufficient size for definite biopsy, the fluorescent areas were completely necrotic and the nonfluorescent areas were composed of viable tumor cells. The mucosal surface of the tumors never fluoresced per se, although both to tumor and to normal mucosa, fluorescent intestinal contents were often adherent. The majority of involved lymph nodes did not fluoresce, either on the intact surface or on cut section. However, in four cases (three carcinomas of the stomach and one carcinoma of the colon) metastatic nodes fluoresced well. In two of these cases, the nodes had been completely replaced by tumor and presented many areas of necrosis. In the other two cases, the nodes were only partially involved with tumor, cut surfaces were very mucinous, and microscopic sections confirmed this secretion of mucin by the cancer cells.

Shapiro and Landing (*6*) injected sarcoma-bearing mice with fluorescein intravenously. They reported that when the interval after injection was under 12 minutes, the fluorescence about the tumor tended to be greater on the periphery, that in from 12 minutes to 3 hours there was uniform marked fluorescence of the tumor, and that from 3 to 7 hours after injection the dye was observed predominantly or only in the areas of necrosis. It should be noted, however, that the dosage (approximately 1.5 gm./kg.) used by these men in mice was nearly 100 times as great as that used by us in humans. Working with patients, it is difficult to correlate staining with a definite time interval, since each tumor varies in regard to necrosis and to the inflammatory reaction in the surrounding stroma. Certainly in our cases it was not unusual for fluorescence to occur simultaneously about the periphery of the tumor and also in the center of the mass, apparently in necrotic areas, at intervals varying from 3 to 11 hours following injection.

Fluorescein is excreted principally by the kidneys and the liver. Thus over a period of approximately 24 hours following injection, the urine and the bile are brightly fluorescent. Due to excretion of the latter, the intestinal contents fluoresce. Although fluorescein, an acid dye, is not excreted by the gastric glands (*10*), the mucus within the stomach often fluoresced, apparently due to regurgitation of bile. In the one case in our series with carcinomatous ascites, the ascitic fluid also fluoresced well.

Discussion

The localization of dyes in relation to animal tumors has been described by numerous men, among them Weil, Ludford, Zahl and Waters, and Duran-Reynals. Weil (*7*), using 18 synthesized Congo Red derivatives, injected tumor-bearing mice intravenously or subcutaneously. He found that the dyes localized usually in the necrotic core of the tumor and that

living tumor cells did not stain with any of the dyes. Ludford (*5*) studied the localization of Trypan Blue in mice with transplanted, spontaneous, and tar-induced tumors. He reported that the necrotic areas of tumors were usually well stained and that deeply stained areas occurred around many tumors due to the taking up of the dye by the macrophages, lymphocytes, and fibroblasts in the periphery of the tumor and in its stroma. Only very rarely was an occasional tumor cell found which was itself stained. In mammary adenocarcinoma, cancer cells were occasionally present in which their secretions were faintly stained, but this staining was much less intense than in the normal mammary gland. Zahl and Waters (*8*), using principally Evans Blue and Lithium Carmine, found, after injection into more than 150 mice bearing either sarcoma 180 or transplanted or spontaneous mammary carcinoma, that the dyes did not localize in the tumor cells but in the stroma surrounding the tumor. The dye first appeared diffusely throughout the stroma but in 24 to 48 hours appeared in the macrophages at the tumor edge. Duran-Reynals (*1*), utilizing various animal tumors, concluded that Evans Blue localized about the tumors more strongly than did Trypan Blue or Chicago Blue. He likewise found that dye did not appear in healthy tumor cells, though an occasional necrotic tumor cell presented a slight diffuse stain.

In contrast to the above, Engel (*2*), working with the dyes, Fuchsin, S., Lichtgrun, and Wasserblau, in relation to a transplantable sarcoma of mice, found that although carcinoma cells were not vitally stained, these sarcoma cells were stained diffusely. Lewis, et al. have reported that Nile Blue and some of its related dyes, when fed by mouth, stain the cells of a transplanted mouse sarcoma (*3*, *4*). It is to be noted that both of these authors describe a diffuse staining of the tumor cell. This is in contrast to the general finding after vital staining that dye appears in healthy cells in the form of globules and that in injured or nonviable cells the dye appears as a diffuse stain (*12*).

Nevertheless, from the work of Weil, Ludford, Zahl and Waters, and Duran-Reynals (*1*, *5*, *7*, *8*), it appears that at least in the case of dyes of larger particle size, such as Evans Blue or Trypan Blue, neoplastic cells are not themselves stained selectively but that the localization of the dye in the region of tumors is based on some other mechanism. Duran-Reynals found that foreign sera localize selectively about tumors in a manner similar to Evans Blue. However, he reported that the more diffusible dyes such as eosin showed no difference in localization in normal and malignant tissues. Such facts at least suggest that the phenomenon of localization is largely a physical one. As concluded by the latter author, it may be due to increased capillary permeability in the area of neoplastic cells. That such an explanation is more likely than a unique chemical relationship between the dyes and the neoplasm is also suggested by the fact that we found localization of fluorescein about such non-neoplastic lesions as benign peptic ulcers or a perforated diverticulum of the colon. Such localization of dyes in inflammatory lesions of non-

neoplastic origin has also been shown experimentally by Moore and Tobin (*13*) and by Kroll, Strauss, et al. (*14, 15*).

It therefore appears that fluorescein, in localizing in the area of a certain number of gastrointestinal neoplasms, does not do so because of any particular property of this dye alone. In fact, it is probable that a dye of larger particle size might show more selectivity for the tumor compared to the rest of the body. However, for clinical use, three characteristics of fluorescein enhance its value. Fluorescein is excreted rapidly (24–48 hours). Relatively large doses may be given without causing marked discoloration of the patient under ordinary illumination. On the other hand, when viewed under ultraviolet light, small amounts of the dye fluoresce brilliantly. In spite of these properties, however, our results suggest that fluorescein does not localize about tumors of the gastrointestinal tract with enough constancy or enough exactness to prove of clinical value.

It should be emphasized that such reports as those of Duran-Reynals (*1*), Weil (*7*), or Zahl and Waters (*8*) state that the liver and kidney also showed fair localization of dye. Weil found that the liver contained as much, if not a greater, concentration of Congo Red and its derivatives than did the tumor. Zahl and Waters stated that although the dye disappeared from the liver in several days, the renal tubule cells remained stained as long as the dye was excreted. Such facts are, of course, of significance should diagnostic or therapeutic procedures be considered, based upon radioactive compounds.

LESIONS OF THE CENTRAL NERVOUS SYSTEM

Kafka (1912) was the earliest worker to use fluorescein as an aid in the study of central nervous system disorders (*11*). He injected the dye into animals and found that all parts of the body were stained with the exception of the brain and spinal cord. It was noted that the choroid plexus was also stained by the dye. He administered large doses of sodium fluorescein by mouth to patients and investigated its appearance in the spinal fluid. He found that it appeared sooner, and in greater amounts, in patients with general paresis than in others with diagnoses of cerebral syphilis, schizophrenia, or arteriosclerosis.

Schönfeld (*16*) in 1921 also investigated the appearance of sodium fluorescein in the spinal fluid and found that when large doses (6–8 gm.) were given orally, a small quantity of the dye passed into the spinal fluid in about 30 percent of normal individuals but more often in patients with syphilis.

Jervell (*17*) observed in 1925 that large amounts of fluorescein appeared in the spinal fluid of 18 patients with meningitis who had been given orally 0.03 gm. of the dye at least 15 minutes before the spinal fluid was removed. These observations were confirmed and extended by several other investigators (*18, 19, 20, 21*).

Bonar and Bailey (*18*) concluded that the fluorescein test "is of value in the differentiation of epidemic cerebrospinal meningitis from other

diseases in which sometimes there are manifestations of meningeal irritation, such as pneumonia, erysipelas, spasmophilia, encephalitis, brain tumors, and tetanus."

More recently, Broman (*22*, *23*) has published the results of extensive investigations pertinent to this subject. He substantiated earlier work showing that there seemed to be a special type of vascular permeability peculiar to cerebral vessels (so-called blood-brain-barrier). It was shown that after the injection of certain dyes, such as Trypan Blue, damaged areas of the brain were colored by the dye while normal brain tissue remained unstained. Broman (*23*) then perfused with Trypan Blue a number of human brains removed shortly after death. It was found that tumors, edematous areas, and areas of demyelinization stained selectively. He concluded, therefore, that disorder of the barrier function was present in these instances.

In this clinic (*24*) it was found that when sodium fluorescein was injected intravenously in patients with tumors of the central nervous system, the tumor tissue exhibited a marked fluorescence when viewed under an ultraviolet lamp several hours later.

This technique has been most useful as an aid in locating subcortical tumors for several reasons: (1) Material removed by needle biopsy can be examined for the presence of tumor in the operating room itself; (2) in cases in which the diagnosis is still in doubt, only the fluorescent areas need be sectioned or smeared and stained in the usual manner; (3) edematous brain tissue fluoresces slightly, and when encountered in the biopsy material, it can be concluded that the tumor approximates the biopsy site. Thus, the surgeon can locate the tumor more quickly without exposing a large area of the cortex. This is of real importance in those patients with cerebral edema since herniation of the normal brain through large dural openings may result in irreparable damage.

In several cases, after the malignant tissue was believed to be completely removed, remnants of tumor tissue have been discovered by examining the residual cavity directly under ultraviolet light.

To date, 91 patients have been examined by this technique. Of 68 cases showing a positive fluorescence of biopsy material, 66 were proved to have tumors by microscopic examination, 1 was diagnosed as negative, and another as an abscess. In 23 instances no fluorescence was observed, and, with 2 exceptions, no tumor was found at operation. The 2 exceptions were an angioblastoma and an astrocytoma (table 2).

TABLE 2.—*Diagnosis of intracranial tumors with sodium fluorescein*

Diagnosis	Fluorescence	Cases
Tumor present	+	66
Tumor present	−	2
Tumor absent (false positive)	+−	[1] 2
Tumor absent	−	21
Total		91

[1] In 1 case abscess present.

Attempts have been made to combine fluorescein with several of the heavier elements in the hope of rendering brain tumors radiopaque to the ordinary roentgenological examination. Although no success has yet been attained, calculations based upon the differential concentration of radioactive diiodofluorescein in brain tumors suggest that such a goal is possible.

One compound studied, tetraiodophthalic fluorescein, has proved to be an efficient agent for intravenous cholecystography (*25*). Other fluorescein derivatives have been found to be too toxic, insoluble, and unstable. At present, other radiopaque compounds possessing chemical characteristics consistent with the theory of the blood-brain-barrier are being synthesized.

Initial studies (*26*) utilizing radioactive diiodofluorescein for the diagnosis and localization of brain tumors preoperatively have been reported. Briefly, the method consists of the intravenous injection of sodium diiodofluorescein in which radioactive iodine (I_{131}) has been synthesized. Two to four hours later the activity over symmetrical areas of the head is measured by a specially shielded Geiger-Mueller counter. The counting rates for each area per unit time can then be compared for localization of the lesion.

Fifty-two patients suspected of having an intracranial tumor have been subjected to this technique. In every case the radioactive localization test has been done without knowledge of the patient's history or physical findings. The results of the tests have been evaluated independently by members of the neurosurgery staff. Summary of 52 cases: 17 correctly localized; in 10, no focus of higher activity could be found and no tumors were found by angiogram, ventriculogram, or craniotomy, even though the preoperative clinical impression was that a tumor was definitely present; in 2 cases the tumor site as determined by the radioactive technique was close to but not exactly at the actual position of the tumor as revealed by surgery; 1 tumor was localized only as to the correct hemisphere; in 13 instances a tumor was proved to be present and was not localized; in 2 cases, a focus was diagnosed, but no tumor was found at operation; 1 patient had a tumor but it was incorrectly localized; an abscess was not diagnosed; and in 1 instance a localization of a cholesteatoma was not made. (See following tabulation.)

LOCALIZATION OF INTRACRANIAL LESIONS WITH RADIOACTIVE DIIODOFLUORESCEIN

Tumors correctly localized	17
No tumor localized, none demonstrated by angiogram, ventriculogram, or craniotomy	10
Correct localization (but vascular or demyelinization rather than tumor)	4
Close to, but not exact localization	2
Located as to hemisphere only	1
Tumor present but not located	13
Tumor localized but not found at operation, ventriculogram, or angiogram	2
Incorrectly localized	1
Abscess not localized	1
Cholesteatoma not localized	1
Total	52

It should be pointed out that many of the cases listed as errors had tumors which could not be detected by our present apparatus. If the tumors were benign, such as an acoustic neuroma or cholesteatoma, and, therefore, failed to take up much dye, or if they were less than 4 cm. in diameter and situated near the base of the brain, not enough radiation would be contributed by the tumor to differentiate it from the surrounding tissue. The greatest difficulty was encountered in the attempt to localize small cerebellar tumors. The easiest tumors to diagnose were the more malignant glial tumors, especially if they were accompanied by some edema and were near the surface of the brain.

In order to evaluate the physical limitations of this technique, measurements were made of conditions simulating those found clinically (*27*). A human calvarium was filled with a solution of radioactive I_{131}, and small glass bulbs (tumors) of various sizes were suspended in the solution at different depths. By varying the conditions, the minimal condition of tumor size, differential concentration of radioactive dye in the tumor, and the counting rates per minute were determined. It was found that if the concentration of dye in the phantom brain tumor was twice that of the surrounding tissue, the minimal volume of a tumor that could be detected was about 40 cc. If greater concentration ratios were present, smaller tumors could be detected.

In order to clarify this point, patients were injected preoperatively with tracer doses of radioactive diiodofluorescein, and biopsies were taken of the tumor and adjacent normal tissue. The activity found in brain tumors (human) ranged from 1.64 to 29 times that of adjacent brain tissue. In general, the highest levels of activity were found in the most malignant tumors (glioblastoma). It should be remembered that the biopsies of normal tissue were obtained adjacent to the tumor and, therefore, often contained more dye than if the tissue could have been removed from a more distant site.

In order to screen various dyes as to their possible use as diagnostic agents and to study the certain effects of radiation, brain tumors were induced in several strains of inbred mice. The technique employed utilized methylcholanthrene and was similar to that reported by Seligman and Shear (*28*). At present, 17 different glial tumors are being used in various experiments. In these tumors, dye concentrations as high as 90 times that of normal brain tissue have been obtained.

Discussion

The reason for the accumulation of certain dyes in brain tumors is not entirely clear. That it is not wholly a disturbance of the so-called blood-brain-barrier seems substantiated by the fact that some gastrointestinal and other cancers also show a similar differentiation. That it is not due to a greater vascularity is obvious from the fact that maximal fluroescence does not occur immediately after injection of the dye but gradually increases in intensity for from 2 to 4 hours. Also, the tumors with the

greatest blood supply (angiomas) are not very fluorescent and, in fact, resulted in one diagnostic error.

The theory that a blood-brain-barrier exists in the central nervous system has occupied the attention of many investigators since it was originally proposed by Goldmann (*29*) in 1913. According to some workers (*22*, *23*), the barrier is a special property of cerebral vessels. This statement, however, should probably be modified by the addition of the retinal vessels of the eye (*30*).

Friedman and his coworkers (*31*, *32*) demonstrated that normally only substances which carry a negative charge are blocked by the barrier. This has been reemphasized by Spatz in 1933 (*33*), and Broman in 1944 (*23*).

Broman (*22*) reported that the barrier exists in the intima of the blood vessels. In experimental animals he apparently damaged the barrier by the injection of noxious substances into the carotid artery but only by application of them to the pia. As mentioned previously, Broman found the function of the blood-brain-barrier impaired or absent in brain tumors, encephalomalcia, edema, abscesses, and multiple sclerosis. With this in mind, it is not surprising that we found a slight fluorescence in areas of edema and that the more edematous areas contain more dye.

Also, it could be predicted that brain abscesses and their capsules would contain more fluorescein. That this did occur in one of our patients was pointed out earlier.

In this regard, an additional interesting clinical observation should be recorded. Since the inititation of the fluorescein studies, three patients with a history of Jacksonian epilepsy have been subjected to craniotomy. In each instance, small cortical areas were found to fluoresce vividly. These fluorescent areas corresponded closely to the site which could be expected to be related to the particular form of epileptic seizure as judged by clinical study. The fluorescent area (10 mm. in diameter) in the first patient (area 8) was biopsied but was not removed. The biopsy was reported as normal. This patient has continued to have seizures. In the other two patients, the fluorescent areas were excised, and although these patients have been free of further seizures, it is still too early to prognosticate the eventual outcome of these cases. The significance of these observations will not be known until they can be correlated with cortical stimulation studies.[3]

Fluorescein is an excellent compound for these purposes because, as mentioned previously: (1) Large amounts can be given without highly coloring the patient's skin; (2) it can be detected in extremely small amounts, when exposed to ultraviolet light; (3) it is an acid chromagen dye, and therefore, can be used to accentuate disturbances of the blood-brain-barrier.

Although they localize in experimental brain tumors, clinical use of

[3] Further observations have revealed that most of these fluorescent areas probably resulted from the use of coagulating current upon the dural vessels above the affected site. This will be reported in more detail in a future article.

other acid dyes such as disulphine blue [4] have been disappointing. Sufficient amounts to produce sharp color differences between neoplastic and normal brain tissue also stain the skin with midnight hues. This circumstance is frightening to the patient, confusing to the anesthetist, and may result in serious medical-legal problems.

At present, it seems doubtful that the blood-brain-barrier mechanism can be utilized for therapy of brain tumors. The most likely therapeutic attack is probably the use of the slow neutron-boron reaction ($_5B^{10} + _0Ns^1 \rightarrow _3Li^7 + _2He^4$) which Zahl (*34*) and others (*35*) suggested some years ago. To this end various compounds containing boron and having characteristics which take advantage of the blood-brain-barrier phenomena are being synthesized and tested in the laboratory at the present time.

SUMMARY

Sodium fluorescein has been shown to concentrate in an unpredictable manner in certain body tumors. Of 36 cases of carcinoma of the stomach, 17 showed an appreciable localization of the dye about the tumors. Only 2 of 15 cases of carcinoma of the colon showed such localization. The most consistent results were obtained in mucinous adenocarcinomas of the gastrointestinal tract and peritoneal implants of generalized carcinomatosis.

The concentration of fluorescein about malignant tumors is not a unique quality of the dye itself since large amounts of the dye can be found in areas of inflammation and necrosis unrelated to malignant processes. It would seem that the observation of fluorescein in tumor tissue is a transient phenomenon dependent upon an increased vascular permeability and a stasis of dye in the tissue itself. When observed at longer intervals after injection, usually only the necrotic areas of the tumors are fluorescent. The role played by changes in the physical aspects of the membranes of malignant cells is unknown but probably is of little significance.

In contrast to use of fluorescein in general body tumors, lesions of the central nervous system concentrate the dye in a consistent and predictable manner. The clinical use of fluorescein to aid in the diagnosis and localization of brain tumors is simple and accurate. It is especially valuable in locating sub-cortical lesions and in differentiating infiltrating tumors from normal brain. In addition, the concentration of the dye in areas of edema, abscesses, and cortical areas associated with certain types of epilepsy, have been described. All these observations can probably be related, in part, to distrubances of the blood-brain-barrier. Other dyes with characteristics which take advantage of the blood-brain-barrier phenomenon have the undesirable property of highly coloring the patient's skin and, therefore, are of little clinical moment.

The clinical value of the use of radioactive diiodofluorescein to diagnose intracranial lesions preoperatively cannot be evaluated at the present time. Although some of the technique's limitation is unknown, better equip-

[4] Imperial Chemical (Pharmaceuticals) Ltd., Manchester, England.

ment and more sensitive Geiger-Mueller tubes or other improved methods for detecting radiation may improve the accuracy and consistency of this test.

Attempts to synthesize radiopaque compounds that would concentrate sufficiently in brain tumors to allow their detection by roentgenography have not been successful. Calculations of the necessary amount of dye per gram of tissue and the necessary differential concentration between the pathological and normal tissue indicate the feasibility of the method.

The possibility of utilizing the slow neutron-boron reaction has been mentioned.

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Chairman Wangensteen: Thank you very much, Dr. Moore. In order to get on with the program, at this juncture, it is important for me to say that the constitution of the program really owes much to my good associate Dr. Morris Barrett who was anxious to assemble for your and my benefit and information as many papers as we could possibly squeeze in. An opportunity may be provided to discuss some of these papers, but I must remind you we have a full program.

The next paper entitled The Metabolism of the Stomach will be presented by Dr. Horace W. Davenport, Professor of Physiology of the University of Utah School of Medicine.

THE METABOLISM OF THE STOMACH[1] [2]

By HORACE W. DAVENPORT, *Department of Physiology, University of Utah College of Medicine, Salt Lake City, Utah*

In the last few years the study of the metabolism of the stomach has moved from the armchair into the laboratory. In the hope of contributing to the solution of the problems of gastric secretion, ulcer formation, and cancer, several groups of scientists have provided the evidence on the metabolism of the stomach which is briefly summarized in this paper.

CARBONIC ANHYDRASE

Carbonic anhydrase, the enzyme which catalyses the reaction $CO_2 + H_2O \rightleftarrows H_2CO_3$, is present in the parietal cells in very high concentration (*1*, *2*). It is now possible to reach a tentative agreement on the role of the enzyme.

The fact of acid secretion has certain consequences, one of which is that hydrogen ions must be supplied to the secreting mechanism. If they are not, either the secreting mechanism must stop, or the interior of the parietal cells must become intolerably alkaline. This idea has been expressed in a number of different ways (*3–6*). The various theories concerning the mechanism of acid secretion which have accompanied the idea have occasionally caused acrimonious polemics, but the differences in the expression of the basic idea are purely formal.

The fact that formation of hydrogen ions was from carbonic acid within the parietal cells offers several physiological advantages. Carbon dioxide is an ubiquitous, uncharged molecule which diffuses freely through tissues. When the appropriate pH difference exists, carbon dioxide is rapidly hydrated to form carbonic acid which in turn ionizes to give one hydrogen ion and one bicarbonate ion. The hydrogen ion could provide the secreting mechanism with the cation it requires, and the bicarbonate ion, by exchanging with chloride from the plasma, could provide the anion of the secreted juice. All the older evidence on the acid-base balance of the gastric blood shows that carbon dioxide does fill this role (summarized in *3* and *5*).

The experimental evidence is that gastric carbonic anhydrase can be inhibited more than 99 percent without inhibition of acid secretion (*7*, *8*). On the basis of his studies of the kinetics of the hydration of carbon dioxide, and supported by reasonable assumptions concerning the conditions prevailing within the parietal cells, Roughton (*9*) has calculated

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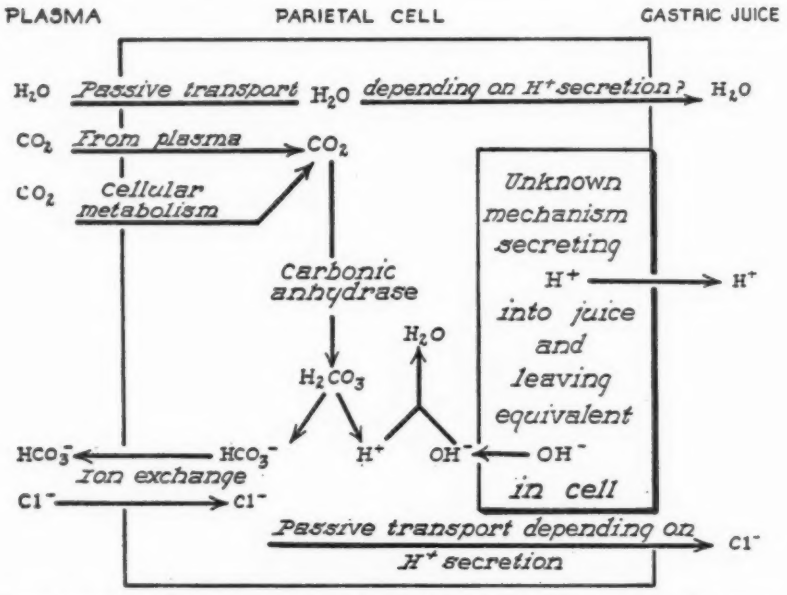
[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] The unpublished work mentioned in this paper was supported by a grant from the American Cancer Society, recommended by the Committee on Growth.

that when the cells are secreting acid at a high rate, the hydration of carbon dioxide must be accelerated over the uncatalysed rate by 15 to 400 times. He has also calculated that there is enough carbonic anhydrase present to accelerate the reaction 15,000 times. It can be concluded, insofar as the calculations are valid, that less than 1 percent of the enzyme is necessary.

The question of the role of carbonic anhydrase is not settled to the satisfaction of all. Conway and his collaborators (*10*) believe that the source of the hydrogen ions secreted is an organic acid such as pyruvic acid which undergoes decarboxylation. They also believe that the product of decarboxylation is carbonic acid and not unhydrated carbon dioxide. If these two beliefs prove to be true, the role of carbonic anhydrase may be to catalyse the dehydration and not the hydration of carbonic anhydrase. (See text-fig. 1.)



TEXT-FIGURE 1.—A tentative scheme describing the role of the carbon dioxide system in the parietal cells.

If a parietal cell secretes more hydrogen ions than it reduces molecules of oxygen, some carbon dioxide must be supplied from exogenous sources. Comparison of the oxygen uptake with acid secretion shows that at least occasionally the ratio of acid-secreted to oxygen-used can be greater than one (*6*).[3] Consequently, it is likely that some of the carbon dioxide used by the parietal cells is derived from arterial blood and ultimately appears in the venous blood as bicarbonate.

[3] GRAY, M., HOKIN, R. E., and REHM, W. S.: Studies on the aerobic glycolysis in the gastric mucosa of the anesthetized dog. Read before the American Physiological Society, Minneapolis (1948).

If alkali, unneutralized by carbon dioxide, is allowed to accumulate, the parietal cells will be damaged. It has been shown in experiments on secreting frog mucosa in vitro that if the mucosa is deprived of exogenous carbon dioxide, a form of ulceration occurs (*11*). It is improbable that under normal conditions in vivo it would be possible to deprive the stomach of exogenous carbon dioxide and thus promote ulceration. However, in experiments in dogs whose mucosae are exposed continuously to acid and pepsin, it has been found that the development of profound metabolic acidosis with a very low plasma carbon dioxide concentration increases the incidence of ulcers.[4] Prevention of the development of metabolic acidosis by intravenous administration of bicarbonate reduces the incidence of ulcers. It appears that the carbon dioxide system in the parietal cells helps to maintain their integrity.

GLYCOLYSIS

The mucosa is capable of converting glucose and some other substrates to lactic acid. Under anaerobic conditions and in the absence of a glycolysable substrate, the mucosa disintegrates physically and chemically. If glycolysis is maintained by the presence of glucose, the mucosa is protected against the effects of anoxia for a considerable period (*12*).

Under aerobic conditions the mucosa of at least some species exhibits true aerobic glycolysis (*12*),[5] a characteristic it shares with the intestinal mucosa (*13*). The mucosa contains the mechanism of the Pasteur reaction, for aerobic glycolysis is less than anaerobic glycolysis (*12*). No evidence relating glycolysis to the secretory functions of the mucosa has been obtained.

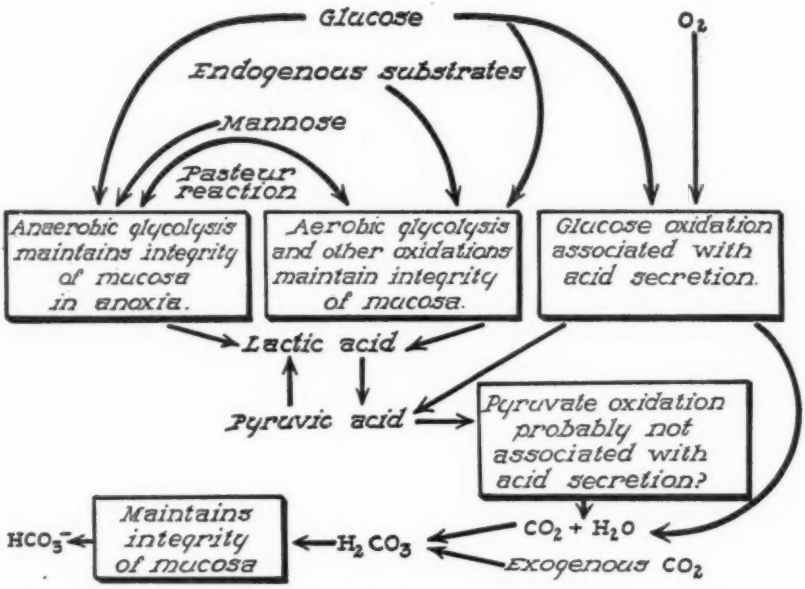
OXIDATIVE METABOLISM

Although the oxygen uptake of the mucosa is the same with or without added glucose, the presence of glucose protects the mucosa from disintegration (*12*). The path by which glucose is oxidized is probably similar to that in other tissues. The mucosa secretes more acid in the presence of glucose than in its absence (*14*), but this may be merely the result of the protective action of glucose. The addition of phosphorylated hexose substrates or glycogen to preparations of the mucosa in vitro neither protects the mucosa nor supports acid secretion (*12*).[5] It is likely that these substrates do not penetrate the cells. There is not a stoichiometric relation between glucose utilization and acid secretion, more acid being secreted in some experiments than can be accounted for by the disappearance of glucose or endogenous glycogen (*6*).[5]

Pyruvic acid is produced, oxidized, and reduced by the mucosa (*15*). Much more acid is secreted than the metabolism of pyruvic acid can account for. Inhibitors of pyruvic-acid oxidation inhibit acid secretion, but not proportionately (*16*). Thiamine is essential for the several

[4] GROSSMAN, M. I.: Personal communication.
[5] DAVENPORT, H. W.: Unpublished observations.

reactions by which pyruvic acid is oxidized, but severe thiamine deficiency does not decrease acid secretion (*17*). Consequently, it can be tentatively concluded that pyruvic acid metabolism is not associated with acid secretion (text-fig. 2).



TEXT-FIGURE 2.—A summary of the reactions known to be involved in the maintenance of the integrity of the gastric mucosa.

Numerous miscellaneous observations have been accumulated concerning the effects of drugs, hormones, amino acids, urea, and other substances upon the metabolism of the stomach (*6, 12, 15, 16*).[6] Histamine increases oxygen consumption in some species in which it also increases acid secretion, but it has no effect on the oxygen consumption of the mouse stomach.

Carbaminoylcholine does not affect oxygen consumption, though it may stimulate acid secretion. Atropine inhibits acid secretion in vitro, but no metabolic effect of the drug has been discovered. Some preparations of urogastrone and enterogastrone may have an effect on the lactic-acid metabolism of the stomach, but the details are still obscure. The stomach deaminates some amino acids at a slow rate, but the amino acids have no relation to hydrochloric-acid secretion, although they must be used in pepsinogen secretion and in the repair of the mucosa. Urea and ammonium chloride have been found to have no effect on acid secretion in vitro.

As this summary shows, there is much to be learned about the metabolism of the stomach. If scientists can avoid blinding their objectivity

[6] DAVENPORT, H. W.: Unpublished observations.

and stultifying their imagination by paternal pride in their theories which, however attractive, must be wrong in some details, we can expect a rapid and fruitful advance in our knowledge.

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Chairman Wangensteen: The next paper, Studies in histochemistry. XX. Urease in the Human Stomach with Respect to Acid Secretion in Ulcer and Cancer, will be presented by Dr. David Glick of the University of Minnesota Medical School.

Dr. Glick: Dr. Davenport has indicated some mechanisms of acid secretion in the stomach and indicated that there are certain lacks in these theories. I don't think we are going to resolve his problem today, but I would like to present some evidence which I think has to be taken into account in the theory of acid secretion: whether it is directly involved or not remains for the future to decide.

STUDIES IN HISTOCHEMISTRY. XX. UREASE IN THE HUMAN STOMACH WITH RESPECT TO ACID SECRETION IN ULCER AND CANCER [1] [2]

By DAVID GLICK, *associate professor of physiological chemistry, Department of Physiological Chemistry, University of Minnesota, Minneapolis*

The possibility that the ammonia and carbon dioxide formed enzymatically from urea are involved in the secretion of acid by the stomach has been considered by various investigators since Luck (*1*) demonstrated the presence of urease in gastric mucosa. In a brief presentation such as this, it is not practical to review all of the pertinent literature, but it should be mentioned that Linderstrøm-Lang and Søeborg Ohlsen (*2*) conducted a histochemical study of the urease in the gastric mucosa of the dog after they were unable to demonstrate any activity in the pig stomach. They reported that ". . . in dogs urease is present in both the pylorus and the fundus region. The enzyme is concentrated in the surface layers of the mucosa where cells stainable by mucicarmine are situated. The chief cells at the base of the glands are inactive in both regions." Later Søeborg Ohlsen (*3*) extended this study to the human stomach. Although urease occurred in the regions of the maximum concentration of parietal cells in the fundic portion, the occurrence of the enzyme in the pyloric area led this investigator to conclude that the enzyme was probably localized in the epithelial and neck chief cells.

The level of the concentration of urea plus ammonia in the gastric juice of dogs was found by Hessel et al. (*4*) to approximate the urea level in the blood, thus suggesting that the ammonia in the juice originated primarily from the blood urea. When the blood urea was increased by nephrectomy, the ammonia plus urea in Pavlov pouch fluid was found to increase proportionally. Although Rigoni (*5*) claimed that the venous blood from the stomach has a higher ammonia concentration than the arterial supply, Mann, Grindlay, and Mann (*6*) could find no increase of ammonia in the venous blood over that in the arterial during acid secretion.

The observations of Mann and Mann (*7*) that certain substances capable of inhibiting urease activity also reduce gastric acidity in dogs are indeed provocative, although these investigators with proper caution point out that their evidence is not sufficient for definite conclusions. The present quantitative histochemical investigation constitutes an attempt to gain some information concerning the relation of the gastric urease activity to acid secretion in pathological conditions, i. e., ulcer and cancer, that result in marked aberrations in the secretory activity.

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] This investigation was supported by a research grant from the National Cancer Institute.

EXPERIMENTAL DATA

Urease Determination: The histochemical method employed for the measurement of urease was that described by Linderstrøm-Lang and Søeborg Ohlsen (*2*). Through the generous cooperation of Dr. O. H. Wangensteen and the members of his surgical staff, the fresh tissue was obtained directly from the operating room. The tissue was spread out in a Petri dish on a piece of filter paper wet with physiological saline solution, immediately frozen at −25° C. and kept at this temperature until the enzyme experiments were set up, usually on the same day.

A cylinder of the frozen tissue, with its long axis at right angles to the mucosal surface, was punched out with a borer having an internal diameter of 5 mm., and then the cylinder was mounted on the freezing head of a rotary microtome so that it was anchored at the muscle end. The sections, 20 μ thick, which were cut parallel to the mucosal surface, were taken serially down to the muscle layer. In the case of the cancer stomachs, malignant cells were diffused throughout the areas examined so that the tissue punch contained cancer cells. The punch was taken near, but not in the gross lesion. Each section to be assayed was individually subjected to the titrimetric procedure, and suitable control experiments were included.

Histological Control: The tissue surrounding the hole left by the removal of the cylinder used for the enzyme determinations was embedded in paraffin, and hematoxylin and eosin stained sections were prepared in the usual fashion. These sections served to establish the cellular composition at various levels from the mucosal surface down to the muscle layer so that a correlation could be made between the enzyme activity and cell types in the manner described by Linderstrøm-Lang, Holter, and Søeborg Ohlsen (*8*).

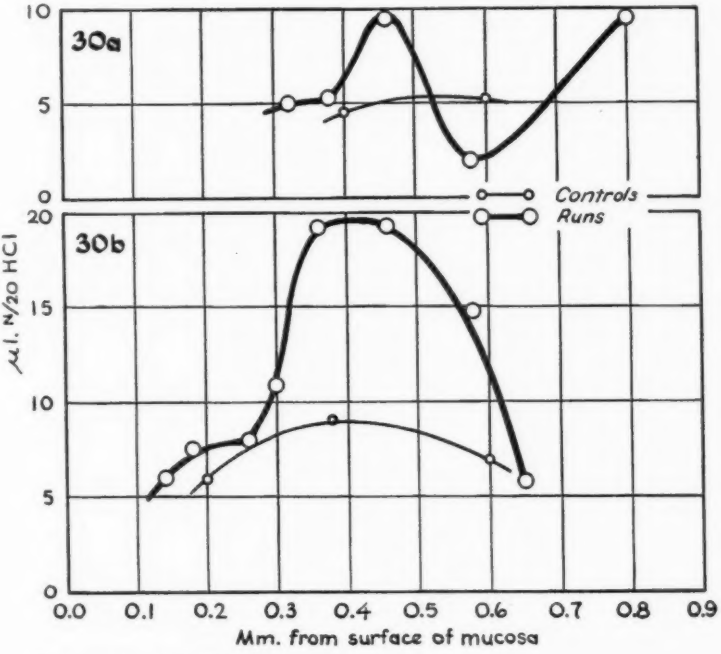
Gastric Acidity: The gastric analyses were carried out by the University of Minnesota Hospital laboratories. The procedure followed routinely is the triple histamine test of Wangensteen et al. (*9*) in which the acid response is measured after each of three separate subcutaneous injections of 0.5 mg. histamine hydrochloride given at 30-minute intervals. Acidities of approximately 30–50° free acid are considered normal responses. The gastric analyses were performed several weeks before the surgery.

RESULTS AND DISCUSSION

The data presented in text-figures 1–3 and 8–9 demonstrate that the antrum [3] portion of the stomachs, whether from normal (esophageal stricture), ulcer, or cancer patients, possess little or no urease activity. The urease activity in the body [4] portion is relatively large when the acid secretory response is more pronounced, as in the normal and ulcer stomachs (text-figs. 1, 4–6), smaller when this response is weaker (text-fig. 7), and virtually absent in the cancer stomachs where there is no response

[3] Designated by "a" after the patient's number on the figures.
[4] Designated by "b" after the patient's number on the figures.

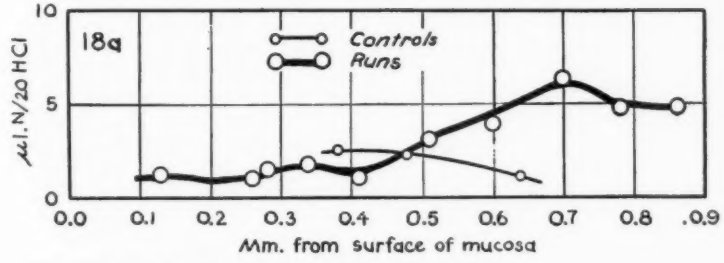
(text-figs. 8–10). The pathological reports given beneath the figures and the interpretation of histological preparations were kindly made by Dr. R. Hebbel of our pathology department.



TEXT-FIGURE 1.—Esophageal stricture.

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	11°	73°	117°	96°
Free acid	4°	59°	100°	80°

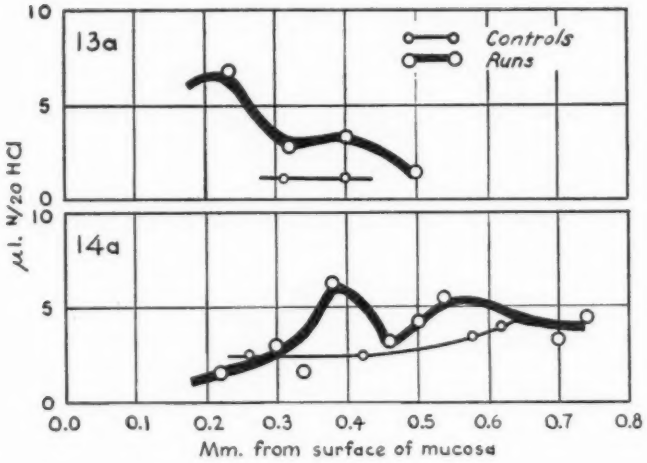
Pathology: Essentially normal; healed erosion on lesser curvature.



TEXT-FIGURE 2.—Congenital esophageal narrowing.

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	37°	61°	78°	69°
Free acid	18°	41°	60°	49°

Pathology: Chronic antral gastritis, celiac ganglion, simple hyperplasia of regional lymph nodes.



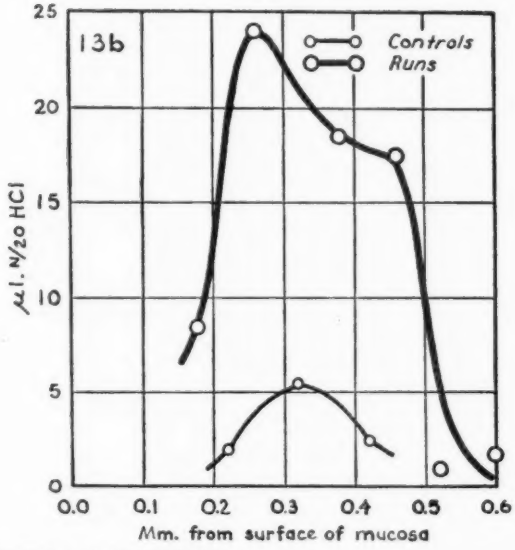
TEXT-FIGURE 3.—Duodenal ulcer.

(13)	Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
	Total acid	121°	124°	138°	140°
	Free acid	0°	10°	36°	48°
	Pathology: Chronic antral gastritis.				
(14)	Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
	Total acid	3°	30°	(Reaction, test stopped)	
	Free acid	1°	17°		
	Pathology: Chronic antral gastritis.				

As in the work of Linderstrøm-Lang and Søeborg Ohlsen (*2*) and Søeborg Ohlsen (*3*), the maximum urease activity was observed in the parietal cell region of the body of the stomach. In the present investigation, urease activity could not be demonstrated in the antral region unless, as happened occasionally, parietal cells were present, a circumstance making it incorrect to consider such tissue true antrum material.

It occurred to Linderstrøm-Lang and Søeborg Ohlsen (*2*) (as it had to Rigoni (*10*), Martin (*11*), and Mann and Mann (*7*)) that urease might be involved in the acid secretion itself, but they were dissuaded from this view by their observations that urease activity was found in the pylorus of the dog, although acid is not secreted in this portion of the stomach, and no urease activity was found anywhere in the pig stomach even though it does secrete acid.

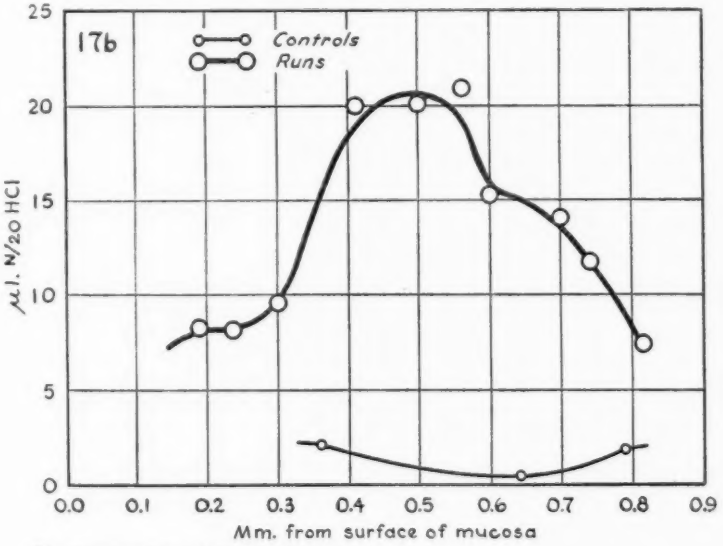
In the light of the findings reported in this paper, it would appear that a certain parallelism exists between the degree of acid secretion elicited by histamine in the human stomach and the intensity of the urease activity. This might be due either to an essential role of the urease in the secretory mechanism or a parallel, but not functionally connected, change in both factors.



TEXT-FIGURE 4.—Duodenal ulcer.

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	121°	124°	138°	140°
Free acid	0°	10°	36°	48°

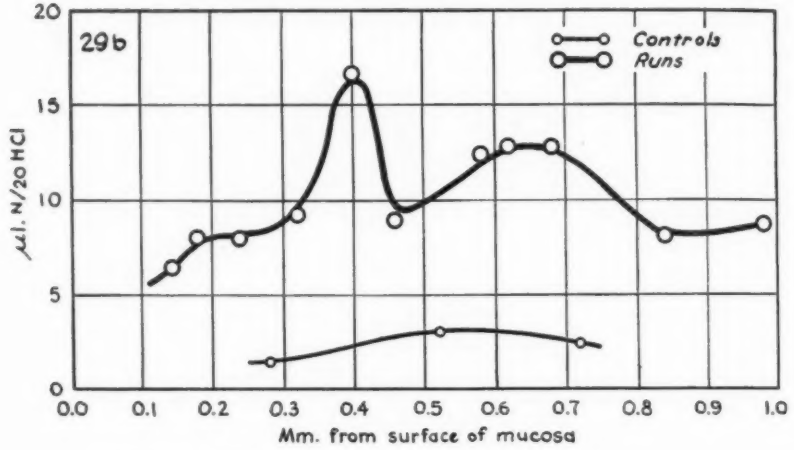
Pathology: Chronic antral gastritis.



TEXT-FIGURE 5.—Duodenal ulcer.

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	52°	48°	82°	68°
Free acid	34°	32°	64°	48°

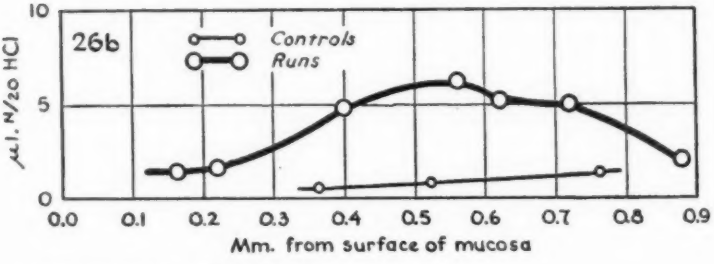
Pathology: Chronic antral gastritis.



TEXT-FIGURE 6.—Duodenal ulcer.

Histamine test.	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	4°	40°	96°	102°
Free acid	0°	10°	66°	72°

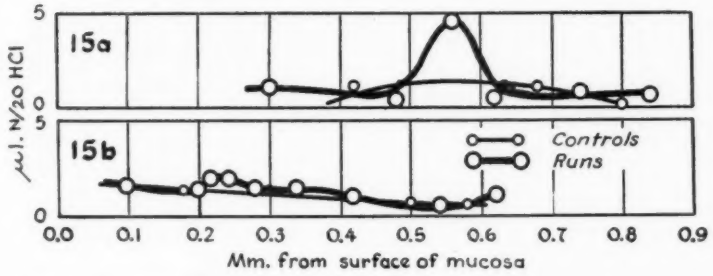
Pathology: Chronic antral gastritis.



TEXT-FIGURE 7.—Duodenal ulcer.

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	9°	26°	35°	43°
Free acid	0°	0°	19°	23°

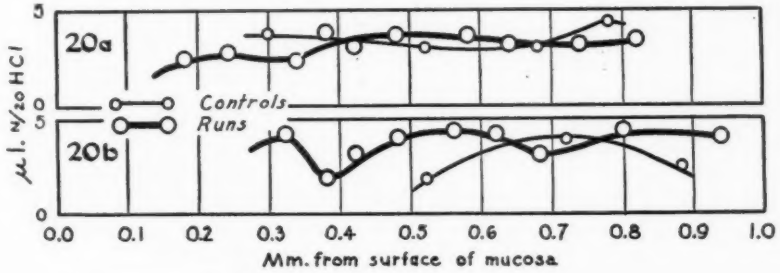
Pathology: Mild chronic antral gastritis.



TEXT-FIGURE 8.—Cancer (pyloric).

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	------	32°	------	(Reaction, test
Free acid	0	0	0	stopped)

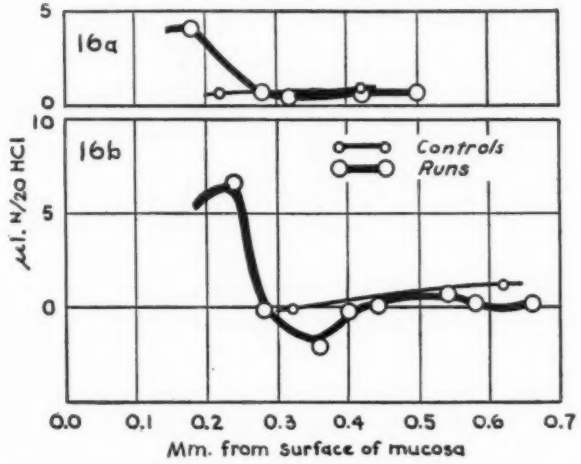
Pathology: Cancer of stomach, regional lymph node cancer, atrophic gastritis.



TEXT-FIGURE 9.—Cancer (pyloric).

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	110°	100°	110°	108°
Free acid	0°	0°	0°	0°

Pathology: Ulcerating adenocarcinoma, atrophic gastritis, simple hyperplasia of regional lymph nodes.

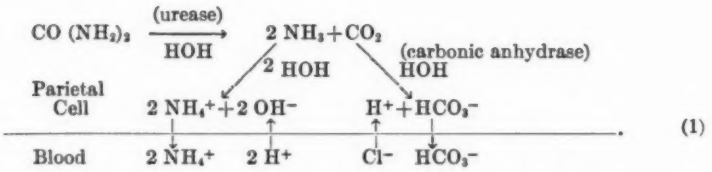


TEXT-FIGURE 10.—Cancer (fundic region).

Histamine test	Fast.	0.5 hour	1.0 hour	1.5 hour
Total acid	8°	10°	13°	14°
Free acid	0°	0°	0°	0°

Pathology: Ulcerating adenocarcinoma, metastatic carcinoma of regional lymph nodes, multiple benign polyp.

This problem is being pursued further, and it would be premature at this time to choose either of the possibilities just mentioned. For purposes of speculation, the following diagram is presented of a scheme which involves the enzymatic decomposition of urea in the formation of hydrochloric acid in the stomach:



(1)

The idea of a urease mechanism to account for the secreted hydrogen ion is now being tested in collaboration with Dr. N. Lifson using as a key in the working hypothesis an exchange of hydrogen ion from the blood for ammonium ion from the urease action in the mucosa. In conjunction with Drs. F. J. Lewis and C. W. Lillehei of the surgery department, studies are also being pursued on the effect of pharmacological and surgical factors which are known to influence acid secretion on the urease activity.

SUMMARY

A quantitative histochemical study of the urease distribution in the human stomach revealed that the antral mucosa from patients with normal stomachs, duodenal ulcer, and gastric cancer had little if any of the

enzyme activity in any of the cases. However, the body mucosa possessed appreciable urease activity which was maximum in the parietal cell region, and the intensity of the activity appeared to be proportional to the acid secretory response elicited by histamine. In the cancer stomach in which no response could be provoked, no urease action was demonstrable.

The possibility of a relationship between acid secretion and urease activity was discussed.

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Chairman Wangensteen: Thank you, Dr. Glick. The next paper is Effect of Intravenous Glucose on Gastric Lactic Acid in Gastric Carcinoma by Dr. Bernard Shacter, Laboratory of Experimental Oncology of the University of California and the National Cancer Institute.

EFFECT OF INTRAVENOUS GLUCOSE ON GASTRIC LACTIC ACID IN GASTRIC CARCINOMA [1]

By Bernard Shacter, Ralph L. Byron, Jr., and Michael B. Shimkin, *of the Laboratory of Experimental Oncology, National Cancer Institute, National Institutes of Health, Public Health Service, and the University of California Medical School, San Francisco*

Following Warburg's (*1*) demonstration of the glycolytic activity of tumor tissue, several investigators, notably Cori and Cori (*2*) and Kahler and Robertson (*3*), found an accumulation of lactic acid in tumors following the administration of glucose to tumor-bearing animals.

It was considered of interest to investigate whether this phenomenon could be demonstrated in man, particularly in patients with gastric carcinoma. Gastric carcinoma is at least partially accessible through a gastric tube, and it was anticipated that increased glycolytic activity of the neoplasm following glucose administration might be reflected by changes in the concentration of lactic acid in the gastric contents.

The occurrence of increased concentrations of lactic acid in gastric juice of patients with carcinoma of the stomach has been noted by many observers.[2] Robertson (*4*) concluded, on the basis of his experiments on gastric lactic acid formation following the administration of a test-meal, that lactic acid is the product of bacterial fermentation and is not produced by the neoplasm. This view has been generally accepted since his report in 1931. The present study indicates that, at least following intravenous glucose administration, gastric lactic acid may result from the glycolytic activity of the tumor.

MATERIALS AND METHODS

The experimental procedure was carried out on most patients [3] a few hours prior to surgery. The diagnosis on all but one case, therefore, was established by microscopic examination of the tissue removed at operation.

The stomach was washed thoroughly either by continual overnight gastric lavage or by repeated introduction and aspiration of a total of at least 1 liter of water. The washing was then discontinued for about 1 hour to allow accumulation of gastric juice, and the first specimen of the gastric contents was aspirated.

The patient then received an intravenous injection of 100 cc. of 50-percent glucose over a period of 2 to 4 minutes. Samples of gastric juice, with as complete an evacuation of the stomach as possible with

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] The literature on lactic acid in gastric cancer is ably reviewed by Robertson (*4*).

[3] We are indebted to Dr. H. Glenn Bell for making available many of the cases.

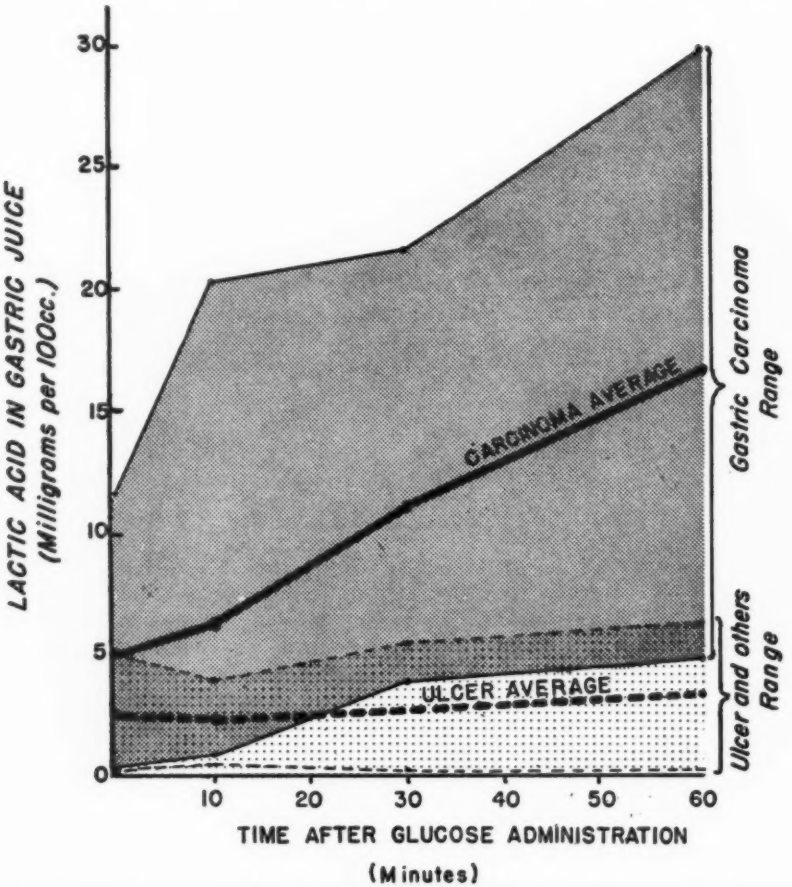
each sample, were obtained 10, 30, and 60 minutes following the intravenous glucose administration.

In several instances, blood specimens were also drawn at approximately the same times as gastric juice samples, for assay of the glucose and lactic acid content. Fluoride was used as an anticoagulant to inhibit glycolysis of blood.

Lactic acid was determined colorimetrically by the method of Barker and Summerson (*5*). The pH of the gastric specimens was determined electrometrically.

RESULTS

The results on 7 cases of gastric carcinoma and on 10 patients with gastric ulcer or other conditions are presented in table 1 and text-figure 1.



TEXT-FIGURE 1.—Gastric lactic acid following intravenous administration of glucose to patients with gastric carcinoma and to patients with gastric ulcer and other lesions (Data from table 1).

In 6 of the 7 cases of gastric carcinoma there was a rise in gastric lactic acid which was evident within one-half hour after glucose administration, and which generally continued during the second half hour. In several cases there was a tenfold rise in lactic acid within 60 minutes after glucose. Case 3E was discrepant in that no appreciable change in lactic acid was observed.

The lowest initial values of gastric lactic acid were observed in patients (4E, 5E, 6E, and 7E) on whom the stomach was washed manually. It is possible that the initial values in the other cancer cases might have been lower if the stomach had been washed manually rather than by the Wangensteen type of lavage.

The magnitude of the rise in gastric lactic acid following glucose administration to patients with gastric carcinoma may be related to the extent of the lesion. Thus cases 2E and 5E, both with large inoperable carcinomas, showed the greatest concentration of lactic acid within 60 minutes.

TABLE 1.—*Gastric lactic acid following glucose*

Case	Sex	Age	Diagnosis	Initial		10 minutes		30 minutes		60 minutes	
				Lactic acid	pH	Lactic acid	pH	Lactic acid	pH	Lactic acid	pH
				mg. per-cent		*mg. per-cent*		*mg. per-cent*		*mg. per-cent*	
1E [1]	M	46	Anaplastic gastric carcinoma	11. 2	7. 4	10. 9	8. 0	16. 7	7. 3	15. 9	7. 8
2E	M	45	Gastric adenocarcinoma	11. 6	----	20. 3	----	21. 9	----	29. 9	----
3E	M	51	Gastric adenocarcinoma	6. 0	2. 6	3. 0	2. 9	5. 5	2. 3	4. 9	1. 8
4E	M	54	Gastric scirrhous carcinoma	2. 2	6. 9	0. 9	7. 4	4. 1	4. 9	12. 4	5. 9
5E	M	81	Gastric polyploid adenocarcinoma	2. 6	5. 2	4. 2	4. 5	12. 5	4. 1	26. 8	5. 4
6E	M	48	Gastric carcinoma, type undetermined	0. 8	7. 3	2. 2	7. 4	11. 2	7. 0	13. 0	7. 4
7E	M	75	Gastric adenocarcinoma	0. 4	3. 6	2. 9	2. 6	6. 5	2. 8	15. 4	5. 2
1C	M	64	Gastric ulcer	3. 2	2. 8	2. 1	2. 5	3. 0	2. 5	3. 5	2. 7
2C	M	26	Gastric and duodenal ulcer	2. 2	3. 7	1. 7	3. 0	1. 8	3. 3	2. 3	3. 6
3C	F	60	Gastric ulcer	2. 1	1. 4	1. 8	1. 5	3. 0	1. 4	6. 4	2. 5
4C	M	59	Gastric ulcer	3. 8	----	1. 9	----	1. 1	----	0. 4	----
5C	F	67	Gastric ulcer	1. 1	7. 2	4. 1	7. 0	5. 6	7. 1	3. 2	5. 4
6C	M	48	Normal [2] (lung carcinoma)	1. 6	6. 5	2. 3	5. 4	2. 9	6. 9	4. 6	7. 1
7C	M	42	Gastric leiomyofibroma with ulceration	3. 0	1. 4	4. 0	1. 4	4. 9	1. 3	5. 5	1. 8
8C	M	58	Gastric ulcer [3]	4. 9	5. 6	3. 9	5. 6	4. 0	4. 5	7. 0	6. 0
9C	M	59	Gastric ulcer	2. 9	2. 3	2. 4	2. 2	3. 0	2. 8	3. 0	3. 8
10C	M	46	Duodenal ulcer	0. 0	1. 9	0. 4	1. 8	0. 0	1. 8	0. 0	1. 9

[1] 50 cc. of 50-percent glucose intravenously; all others received 100 cc.
[2] Autopsy finding.
[3] Clinical diagnosis only.

None of the patients without gastric carcinoma, including one with a benign tumor (7C), showed appreciable rise in gastric lactic acid following glucose injection. The lack of rise in gastric lactic acid in patients without gastric cancer is not correlated with high gastric acidity, since three of the cases (5C, 6C, and 8C) had gastric acidities comparable with those of the carcinoma patients.

From text-figure 1 it is apparent that the average values of gastric lactic acid in the carcinoma cases are appreciably higher than the corresponding

values of the control group. There is no sharp demarcation between the two groups, however, the highest values of the controls overlapping the lowest values in carcinoma.

In patients on whom blood lactic acid determinations were carried out, no differences were observed between the cancer and the noncancer groups. For two bloods on cancer patients, receiving 50 cc. of 50-percent glucose, the whole blood lactic acid concentrations were 9.5 and 12.6 mg. per 100 cc. initially, and 10.6 and 13.7 mg. per 100 cc., respectively, 60 minutes following glucose administration. For four bloods from ulcer patients, receiving 100 cc. of 50-percent glucose, the blood lactic acid levels ranged from 10.9 to 13.2 mg. per 100 cc. initially, and from 14.4 to 17.0 mg. per 100 cc. 60 minutes following glucose administration.

However, in one case of inoperable gastric carcinoma not recorded in table 1, where 1,000 cc. of 5-percent glucose were administered intravenously over a period of 1 hour, the blood lactic acid rose from a fasting value of 11.9 mg. per 100 cc. to 29.7 mg. per 100 cc. 1 hour after the end of glucose administration. Blood glucose determinations, carried out on the same blood samples, showed 122, 522, and 198 mg. per 100 cc. on the initial specimen, at the end of glucose administration, and 1 hour after glucose, respectively.

DISCUSSION

There has been a considerable controversy regarding the origin and significance of gastric lactic acid in gastric carcinoma. Robertson (*4*) demonstrated that most of the lactic acid present in unwashed stomachs of patients with gastric carcinoma is a product of bacterial fermentation. In thoroughly washed stomachs the lactic acid content was low and remained low for several hours. In the present investigation, where determinations were carried out on unwashed fasting stomachs, regardless whether carcinoma was present or not, considerably elevated values of lactic acid were obtained, especially when the gastric pH was high. Most of the lactic acid present in unwashed stomachs may, therefore, be ascribed to bacterial fermentation.

The question still remained, however, whether the neoplasm also contributes to the lactic-acid content of the stomach in gastric carcinoma. To test this possibility, Robertson (*4*) used an oatmeal test-meal and found no rise in gastric lactic acid following the meal. He therefore concluded that all of the gastric lactic acid was bacterial in origin. The test-meal technique is not conclusive, however, since no added metabolic stress is probably placed on the tumor. An increased concentration of glucose must actually be presented to the neoplasm if augmented glycolysis is to be expected. Warburg (*1*) found that the rate of glycolysis of surviving tumor tissue was increased with increasing glucose concentration, while Cori and Cori (*2*) showed that in the fasting state the lactic acid content of mouse tumors approximated that of other tissues of the animal. It was only after glucose was administered and a rise in the glucose content of the neoplasm occurred that lactic acid accumulated in the neoplasm.

In contrast to the behavior of tumor tissue, the lactic acid content of the liver was not altered by glucose injections.

From the results obtained in the present study, it is apparent that increased glycolytic activity in gastric carcinoma may take place following glucose administration, as evidenced by an increased concentration of lactic acid in the gastric juice.

Since there is overlap in the values obtained for carcinoma as compared with noncarcinoma stomachs, and since an increased concentration of gastric lactic acid is not always obtained following glucose administration to patients with gastric carcinoma, it would appear that the procedure used is of little, if any, diagnostic significance. As Robertson (*4*) points out, gastric juice, being a complex mixture of gastric secretions, regurgitant duodenal contents, and secretions from the mouth, throat, and nasopharynx, does not lend itself well to exact and quantitative interpretation. It is known, for example, that jejunal mucosa has glycolytic activity similar to that of neoplasms (*6*). Should jejunal contents reflux into the stomach, an increased concentration of lactic acid might be expected. This, indeed, was found in one case in the present study not included in table 1. This patient had a healed gastrojejunostomy and showed a marked rise in the gastric lactic acid following glucose administration, from an initial value of 1.4 mg. per 100 cc. to 15.4 mg. per 100 cc. 60 minutes after glucose injection. At the same time the pH rose from 4.3 to 6.7, indicating probable regurgitation of jejunal contents. Great caution must therefore be exercised in the interpretation of gastric juice assays.

It was noted that the change in the lactic-acid content of the stomach following glucose administration to patients with gastric cancer was more marked as the stomach was washed more completely and as the amount of glucose administered was increased. This investigation does not preclude the possibility, therefore, that sharper differentiation, of possible diagnostic significance, may be obtained between gastric carcinoma and noncarcinoma patients as better methods of lavaging the stomach, perhaps after obstructing the pylorus, are introduced, and as larger amounts of glucose are administered.

SUMMARY

Glucose was administered to 7 patients with gastric carcinoma and to 10 control patients with gastric ulcer and other lesions, and the effect on the lactic acid level of the gastric juice was determined.

Gastric lactic acid increased significantly in 6 of 7 patients with gastric carcinoma, presumably because of increased tumor glycolysis. No significant rise was observed in nongastric carcinoma cases.

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Chairman Wangensteen: Thank you, Dr. Shacter. The next paper, The Inhibition of Gastric Secretion in Dogs by Human Gastric Juice and Gastric Mucin, will be read by Dr. Charles F. Code, Mayo Clinic, Rochester, Minnesota.

THE INHIBITION OF GASTRIC SECRETION IN DOGS BY HUMAN GASTRIC JUICE AND GASTRIC MUCIN [1]

By CHARLES M. BLACKBURN, M. D., *Division of Medicine,* and CHARLES F. CODE, M. D., *Section on Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minn.*

Substances of biologic origin which depress the secretion of gastric juice have been obtained from three different sources. The first, enterogastrone, is derived from the mucosa of the upper part of the intestine and has been extensively studied by Ivy and his coworkers (*1*). The second, urogastrone, is extracted from urine, and has been especially investigated by Sandweiss and his associates (*2*). The third, still unnamed, was found 10 years ago by Brunschwig and his collaborators (*3*) in achlorhydric gastric juice from human beings. The last is of particular interest to this conference because it was found to occur with great regularity in achlorhydric gastric juice of patients with cancer of the stomach.

Brunschwig and his coworkers tested for this inhibitor by injecting the neutralized juice or alcoholic precipitates of the juice intravenously into dogs with gastric pouches while these pouches were secreting in response to frequent feedings of meat. They noted the presence or absence of depression of gastric secretion after the injection.

Using a quantitative method for the determination of gastric secretory inhibitor activity developed by us in collaboration with Dr. George B. Livermore and Dr. Henry V. Ratke (*4*), we have confirmed and extended some of the observations of Brunschwig and his coworkers. We have found that alcoholic precipitates of achlorhydric gastric juice from human beings inhibit in a striking manner the gastric secretory response of Heidenhain pouches to histamine. The inhibition of histamine-provoked secretion suggests some action of the inhibitor in the gastric mucosa rather more strongly than the inhibition of secretion stimulated by feeding, since the latter conceivably could be due to interruption of digestion and motility.

In attempts to identify more precisely the inhibitor substance or substances in gastric juice, we have found that the activity is associated with the mucous fraction of the juice. Tests of preparations of pepsin have uniformly shown no inhibitor activity. Commercial hog mucin, on the other hand, has consistently shown a high degree of inhibitor activity. Insufficient data are available at present to enable us to state whether or not this gastric secretory inhibitor activity which is associated with gastric mucus is of importance in physiologic or pathologic mechanisms.

[1] Abstract of paper presented before Fourth Conference on Gastric Cancer, San Francisco, December 13, 1948.

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Chairman Wangensteen: Thank you, Dr. Code. The next paper is to be read by Dr. Franklin Hollander and is entitled Histological Study of the Destruction and Regeneration of the Gastric Mucous Barrier Following Application of Eugenol. Preliminary Report.

HISTOLOGICAL STUDY OF THE DESTRUCTION AND REGENERATION OF THE GASTRIC MUCOUS BARRIER FOLLOWING APPLICATION OF EUGENOL. PRELIMINARY REPORT[1][2][3]

By FRANKLIN HOLLANDER, Ph. D., and RHODA L. GOLDFISCHER, A. B., *Gastroenterology Research Laboratory, The Mount Sinai Hospital, New York*

INTRODUCTION

At the Gastric Cancer Conference in 1946 (*1*), our group of investigators reported on the changes in character of gastric mucous secretion which result from the repeated application to the gastric mucosa of dogs of a mucigogue-desquamating agent. This physiological study had been conducted with Heidenhain-pouch dogs, using an aqueous emulsion of eugenol as the stimulus. During the last year, these experiments were repeated with this modification: the experiments were terminated at various stages of progress by killing the dogs, in order to study successive histological changes in the surface of the gastric mucosa. The present report is a preliminary statement concerning the results of these experiments. The immediate objectives of this line of investigation are: (1) to trace the extent of functional exhaustion and recovery of the mucous secreting cells; (2) to determine the extent of desquamation of the surface epithelium; and (3) to ascertain the changes in character and the extent of replacement of the mucous cells themselves. In addition, these studies promise to yield information on the cytological processes of mucous secretion in the stomach and, possibly, evidence concerning the early stages in the experimental development of gastritis.

The broad plan of the original physiological experiments is as follows: after a considerable control period, a 5-percent aqueous eugenol emulsion was placed in the pouch for 15 minutes and then aspirated completely, following which four 30-minute specimens of secretion were collected. The dog was allowed a rest period of about 15 minutes, and then a second cycle of stimulation and collection was started. These cycles were repeated until the secretion had changed in character from a true viscous mucus to a fluid which was mostly inflammatory exudate, i. e., until it was inferred that the mucous barrier was completely exhausted. Although this change usually required five successive cycles, an additional one or two cycles were added to insure completeness of the transformation in

[1] Presented before the Fourth Conference on Gastric Cancer, held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] This investigation was supported by a research grant from the National Cancer Institute, National Institutes of Health, Public Health Service.

[3] The authors wish to acknowledge the participation of Dr. B. P. Sonnenblick in the early part of this investigation.

character of the secretion. Thus, these fatigue experiments entailed six or seven cycles in all and a duration of 15 to 18 hours. The animal was then returned to its cage for a recovery period of 30–36 hours after which the eugenol was again applied as before, until the mucus-secreting apparatus was once more fatigued. This first follow-up experiment, designed to reveal the extent of tissue recovery which occurred during 1½ days of rest, usually lasted for only one or two cycles before the secretion again became a bloody serous fluid. Subsequently, a second follow-up experiment was performed, but this time the intervening recovery period lasted 2 to 3 months. As a result, it then required three or four cycles to fatigue the mucous barrier, indicating that this structure had recovered extensively, but it had not yet returned to its original state prior to the very first application of eugenol. On the basis of the changes in several physicochemical properties of the secretion and in the microscopic appearance of smears from the numerous specimens collected in the course of these studies, inferences were drawn regarding the progressive changes which had probably occurred in the gastric mucous barrier. In order to confirm these inferences, the present series of experiments was undertaken.

EXPERIMENTAL PROCEDURES

The general plan of the present experiments, as well as the details of technique, were essentially the same as before (*1*). This time, however, the individual experiments were interrupted at various stages by killing the animals. Pieces of tissue were then removed from pouch and residual stomach and placed in Bouin's fixative as rapidly as possible. Sections were stained with haematoxylin and eosin and with toluidine blue. A total of 19 pouch dogs were prepared for this study; of these, 1 died, and 2 were discarded for reasons unrelated to the project. None of the dogs was ever used for any purpose but this, and a period of 2–3 months was always allowed to elapse between preparation of the pouch and the initial fatigue experiment. In addition to the residual stomachs of all the animals, 4 of the pouch dogs served solely as controls and were killed 2 to 3 months after operation without having received any eugenol treatment whatever. The initial fatigue experiments were terminated at the following approximate times after removal of the stimulus: ¼, ½, 1¼, and 2¼ hours following a single application of eugenol; ½, 1½, and 2 hours following the third such application; and 2 and 36 hours after the seventh. In addition to these dogs which were killed in the course of the initial fatigue series, there were 3 which were subjected to subsequent follow-up experiments. One of these animals was sacrificed 3 months after the first follow-up experiment had been terminated. The other 2 dogs were subjected to a second follow-up experiment 5 months after the first; these were allowed final recovery periods of 3 weeks and 4 months, respectively, and were then killed. One to 8 pieces of the residual stomach were taken from all 16 animals for control purposes, in addition to specimens from the pouches of the 4 untreated dogs. Thus, by varying the number of eugenol applications and the time which was allowed to elapse between the last

such application and the death of the animal, it was possible to study a variety of stages of impairment and recovery of the mucous barrier. The following are the results as revealed by some of the more obvious differences in structure of the tissue surface.

RESULTS

Controls

Sections of untreated tissue (Pl. 56, fig. 1, CA 75–8) generally reveal surface mucus as very thin short ribbons or small amorphous masses of metachromatic material, interspersed with areas of normochromatic substance. The crypts contain much less mucus than is present on the surface of the mucosa, and in both regions the material is nearly, if not entirely, free of columnar cells. In depth, the crypts may be long or of only moderate size relative to the length of the entire gastric gland, and these two varieties occur with about equal frequency. There are virtually no stretches of mucosa entirely free of crypts, and only rarely does one encounter a shallow crypt or a depression in the surface suggestive of the remnant of a foveola which had been removed by desquamation of its columnar epithelium. In fact, the control specimens give almost no evidence whatever of active desquamation; even gaps in the rows of columnar cells, indicative of cells which have been shed and not yet replaced, are encountered only rarely, and these are usually no more than one cell wide. Ranks or palisades and single columnar cells are seen within the adherent layer of surface mucus only occasionally, if at all. Finally, both within the crypts and on the surface of the mucosa between the crypt mouths, practically all of the mucous cells are tall columnar cells with large thecae containing metachromatic material. Occasionally a parietal cell, white blood cells, or a few erythrocytes may be seen wedged in or just beneath this layer of surface epithelium.

Destruction of the Mucous Barrier

Successive stages in the degenerative process were traced by three experiments which were terminated in the first, third, and seventh cycles of the fatigue series, respectively. In the first two of these experiments, ½ hour elapsed following removal of eugenol; in the last, this time interval was 2 hours.

A quarter of an hour following the first application of eugenol (Pl. 56, fig. 2, CA 75–2), the pouch sections already gave evidence of significant changes from the controls, and this observation is supported by the over-all picture at the end of the first half hour (Pl. 56, fig. 3, CA 76). Mucus in considerable amounts is extensively distributed over the surface, and even within the crypts, though here again to a lesser degree. Although some of this mucus is acellular, especially in the crypts, most of it contains numerous columnar cells, singly and in ranks, and also some parietal cells, neck chief cells, white blood cells, erythrocytes, and other elements which have already been reported as occurring in mucous smears. Although the pro-

portion of short crypts is about the same as in the controls, really deep ones are rarely encountered, whereas shallow depressions in the mucosal surface are notably present. Furthermore, there are now a great many more gaps between the columnar cells than were found in the untreated specimens, and these spaces are often six or more cells in width. There can be no doubt that these gaps result from desquamation rather than technical defects, because large and small ranks of columnar cells are dispersed in the extracellular mucus immediately adjacent to them, and palisades of these cells can often be seen in the process of being cast off. In short, these two experiments give ample evidence of a progressive increase in both secretory and desquamatory responses during the first ¾ of an hour of cycle A in the fatigue experiment.

In the third cycle (Pl. 56, fig. 4, CA 64), 30 minutes after removal of the eugenol, the destruction of the mucous barrier is seen to have progressed even further. The amount of extracellular mucus is now markedly decreased, and its cell content somewhat less so, but signs of active desquamation are still present. This secretion is only slightly metachromatic and it contains some exudative material with traces of blood. The proportion of surface which is crypt-free or contains only shallow depressions is even greater than before, and the gaps are now sufficiently wide and numerous as to suggest actual denudation with exposure of the underlying connective tissue. Although tall columnar cells with metachromatically staining thecae are still present, there are now numerous columnar cells which are theca-free and also some flat cells not encountered in the experiments described above. These cells will be considered in detail below.

Finally, by the end of the seventh cycle (Pl. 57, fig. 5, CA 68) definitely formed crypts have disappeared to a very large extent. Shallow depressions predominate, though regions entirely devoid of crypts are also numerous. The surface of the tissue appears to have lost most of its mucus-containing columnar cells, and there are now many regions which are completely denuded of surface epithelium. It is only in the small proportion of remaining crypts that metachromatically staining columnar cells still occur. Signs of active desquamation are virtually absent, and the surface is now covered with a bloody inflammatory exudate containing very little metachromatic mucin and very few mucous cells. Parietal cells are also seen in this extracellular layer.

In this way we have traced the progressive destruction of the mucous barrier through seven cycles of the fatigue experiment, to the stage where true mucus secretion and desquamation no longer occur. In general, the histological evidence of this destruction is in accord with the changes in character of the mucus secretion which were encountered in our previous work.

Regeneration of the Mucous Barrier

Our earlier physiological experiments gave evidence of a recovery process as well as a destructive one. Therefore, we next studied the course of

events in three groups of dogs, in each of which all the animals received the same number of 15-minute applications of eugenol emulsion but were killed at different times after the end of such treatment. These three groups consisted of four animals killed in the course of the first cycle of the fatigue experiment, three during the third cycle, and five dogs after the end of a fatigue or of a follow-up experiment.

The existence of a reparative process and the speed with which it occurs are well illustrated by the group of four experiments which were terminated during the first cycle at approximately ¼, ½, 1¼, and 2¼ hours after removal of the eugenol. As noted previously, there was no great difference in the character of the surface epithelium of the first two of these experiments (CA 75 and 76). The epithelial elements present in the surface layer are tall columnar cells, with an occasional parietal and neck-chief cell in or immediately beneath the surface. However, there is one suggestive difference between these two experiments not shown in the photomicrographs. The proportion of mucosal surface that was free of crypts or contained only shallow depressions was less in the half-hour experiment than in the quarter-hour one. Further evidence of this was manifest at the end of an hour (Pl. 57, fig. 6, CA 50) when complete crypts occupied a major part of the entire surface, whereas the proportion of regions with shallow or no depressions was greatly diminished. This reappearance of crypts was itself indicative of a reparative process, but, in addition, the gaps between columnar cells occurred much less frequently than in the two earlier experiments of this cycle and were generally of smaller size.

The mechanism of this reparative process is evidently linked to the appearance of new cells in the surface layer, fusiform and flat epithelial cells which contain no metachromatic material, even though nearby columnar cells have already started to regenerate their intracellular mucus. Flat cells occurred for the first time in the last-mentioned experiment usually in small groups on the surface of the mucosa. Frequently, they occurred at the end of a row of columnar cells which ranged in size progressively from a tall variety through a series of shorter columnar cells, and finally ended in these flat elements. Not only do the cells in these transition series vary in over-all height, but they may also show a gradation in regard to their content of intracellular mucin, from large metachromatic thecae at one end of the series to theca- and mucin-free cells at the other. The presence of even an occasional one of these transition series 70 minutes after eugenol treatment is highly suggestive of a resurfacing of exposed mucosa with newly formed cells, which takes place with amazing rapidity (as reported by Grant (*2*)) probably in advance of crypt reformation. The fact that both mucus secretion drained from the pouch during this time (specimen 4 of the cycle) and extracellular mucus which remained adherent to the mucosal surface in these tissue sections contained significant numbers of columnar cells may be indicative of continuing desquamation in spite of a simultaneous regenerative process. It seems more likely, however, that all traces of eugenol emulsion would have been carried off from the surface of the mucosa by the highly cellular mucus formed during

the first half hour of secretion and that most of the shed epithelium would have been loosened from its basement membrane before the time when this experiment was stopped by sacrifice of the animal. Therefore, the desquamated columnar cells found at this stage of the cycle must have been shed for the most part during an earlier stage. They remained adherent to the mucosa because of the high viscosity and adhesiveness of the mucus in which they were embedded.

One hour later, that is, at the end of the first cycle and about 2 hours after removal of the eugenol (Pl. 57, fig. 7, CA 49), the tissue sections give evidence of still further diminution in mucus output. Also, the content of desquamated columnar cells in this mucus was almost negligible. The proportion of mucosal surface which was devoid of complete crypts was less than in the previous experiment, and extensive denuded regions no longer occurred. Gaps between columnar cells were still present, but their incidence and size were no greater than in some of the control specimens. Thus, although reformation of crypts is still very far from complete, the denuded areas were again covered by some form of epithelium. As in the previous experiments, tall columnar cells predominated, but their average thecal size and mucus content now appeared to be greater. Flat cells were also present in small groups and in transition series, giving evidence that resurfacing of the mucosa was still going on actively at this time.

These regenerative phenomena were all observed in the first group of animals, all of which received only one application of eugenol. The other two groups of experiments (one after three applications, the other after complete fatigue) revealed even greater incidence of these flat cells than was encountered in the experiments just discussed. This was particularly marked at certain stages of the third and seventh cycles (figs. 4 and 5, CA 64 and 68) wherein denudation followed by resurfacing was noticeably more extensive than in the first cycle. Thirty-six and a half hours after the seventh cycle (Pl. 57, fig. 8, CA 69), resurfacing of the mucosa with tall columnar cells appeared to be entirely complete; flat cells were no longer evident in any of the sections. These columnar cells generally contained thecae which were relatively large and full of deeply metachromatic mucus. Crypt reformation also made considerable progress, for although the entire surface was void of crypts 2 hours after the seventh application of eugenol (Exp. CA 68), areas free of fully-developed crypts constituted less than half of the surface after 36½ hours of recovery (Exp. CA 69). It is significant also, that there were more short crypts at this time than long ones, and they all tended to be irregular in shape and even tortuous. Follow-up experiments with recovery periods of 3 weeks to 4 months (Exps. CA 42, 43, and 44) manifested continuing regeneration of the mucous barrier. In fact, when the interval of recovery was 3 to 4 months, the regeneration appeared to be complete, for crypt-free areas were virtually absent.

Even though physiological activity may be restored during the two recovery periods following complete destruction of the mucous barrier,

such recovery of secretory function is not necessarily complete, and this is generally corroborated by the extent of structural recovery found in the present investigation. Thus, in the first follow-up experiments, only one or two cycles were required to effect a radical change in the character of the secretion, from a true viscous mucus to a serous exudate, and in the second follow-up experiments, four cycles were usually required to attain the same end result. In the present morphological study, tissues taken at a time corresponding to the beginning of the first follow-up experiment (1½ days after complete fatigue, Exp. CA 69) revealed only partial replacement of crypt structure, as stated in the previous paragraph. On the other hand, specimens taken 3 months after the first follow-up experiment (Exp. CA 44), which time corresponds to the beginning of the second follow-up experiment, showed an almost completely reconstructed mucosa. Thus, the explanation originally postulated to account for the difference between the first and second follow-up experiments, in regard to the number of cycles required for exhaustion following recovery periods of different durations, is now substantiated by the variations in mucosal regeneration seen in the present study.

CONCLUSION

The histological study, of which this is a preliminary report, presents evidence of the changes in the gastric mucous barrier which result from the repeated application of a mucigogue-desquamatory agent (aqueous eugenol emulsion) to the mucosa of Heidenhain pouches in dogs. In general, the histological evidence is in accord with the results of our previous study on the changes in microscopic and physicochemical character of the mucus secretion collected in the course of such experiments. This correlation of the physiological and histological observations obtains with regard to the impairment and virtually complete destruction of the mucous barrier and also with regard to its subsequent regeneration. Successive applications of the eugenol emulsion effect a progressive removal of the columnar cells from the surface of the mucosa and then from the crypts themselves, until the connective tissue matrix containing the collecting tubules of the glands is exposed. The regenerative process can be divided into three stages: (1) a preliminary resurfacing of denuded mucosa with flat and fusiform-shaped cells, which is already evident 30–60 minutes after removal of eugenol from the pouch; (2) the transformation of these new cells into low and tall columnar cells; and (3) the reformation of crypts in these areas of smoothly resurfaced mucosa. This entire process has been found to occur within 36 hours following the removal of the mucosa as far down as the bottoms of the foveolae. The surprising rapidity with which these changes take place serves to explain many aspects of the original physiological experiments which were not fully understood before. Data from these experiments indicate the conditions of pretreatment of the gastric mucosa with eugenol which are most suitable for attempts at carcinogenesis by topical application of known carcinogens after impairment of the mucous barrier. We already know the optimum duration of eugenol

treatment and of posteugenol recovery in order that (1) the protective mucous barrier be impaired extensively but not completely, and (2) that resurfacing of the mucosa be active so as to insure the presence of newly formed cells, presumably in a state of maximum susceptibility to cancer induction. Experiments on rats and mice, analogous to these on dogs, are now in progress, and as soon as they have been completed, carcinogenetic experiments will be initiated.

The experiments described present many problems which require further minute study of the tissue sections. Among these are: the types of cell and mechanism involved in the resurfacing process; the importance of mitosis in the formation of new cells for reconstitution of the mucosa denuded of its crypts and surface columnar cells; the possible occurrence of a progressive gastritis as indicated by lymph follicles, cysts, and tortuous crypts; and the cellular process of mucus secretion, apart from the concomitant desquamation. These are problems for a histopathologist rather than a physiologist, and we now plan investigation in collaboration with such a specialist.

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(*1*) Hollander, F., Sonnenblick, B. P., and Sober, H.: Experimental impairment of the gastric mucous barrier in dogs. J. Nat. Cancer Inst. 7: 361–364 (1947).

(*2*) Grant R.: Rate of replacement of the surface epithelial cells of the gastric mucosa. Anat. Rec. 91: 175–185 (1945).

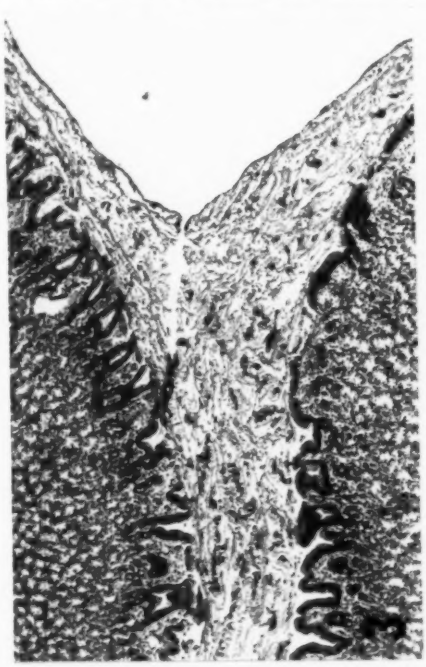
Plate 56.

Figure 1.—Exp. CA 75–8. Control tissue. Residual stomach. No eugenol application. × 100.

Figure 2.—Exp. CA 75–2. Initial fatigue series. Pouch tissue 14 minutes after first eugenol application. × 100.

Figure 3.—Exp. CA 76–1. Initial fatigue series. Pouch tissue 29 minutes after first eugenol application. × 100.

Figure 4.—Exp. CA 64–5. Initial fatigue series. Pouch tissue 27 minutes after third eugenol application. × 100.



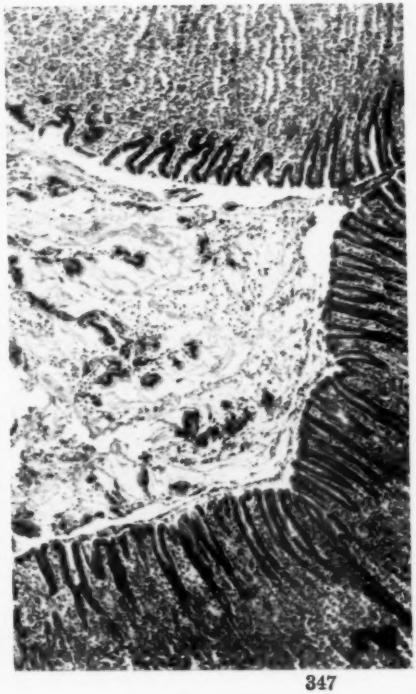


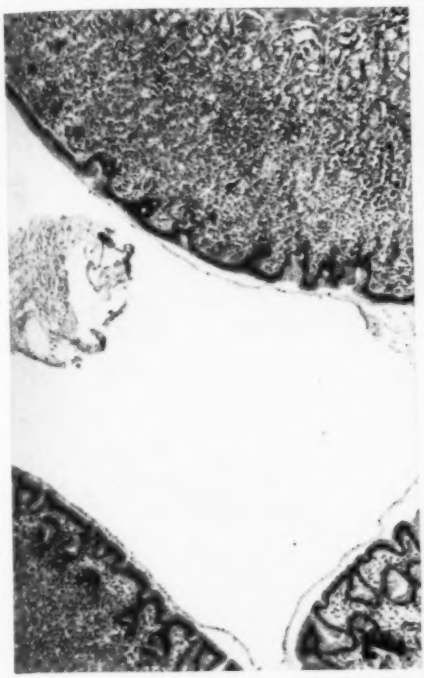
PLATE 57

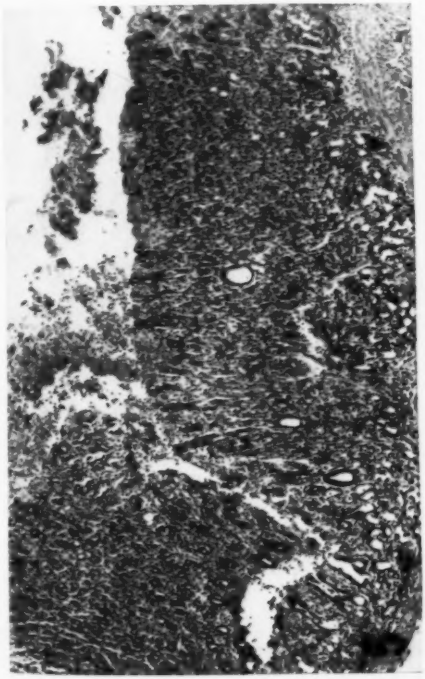
FIGURE 5.—Exp. CA 68–1. Initial fatigue series. Pouch tissue 120 minutes after seventh eugenol application. × 100.

FIGURE 6.—Exp. CA 50–2. Initial fatigue series. Pouch tissue 70 minutes after first eugenol application. × 100.

FIGURE 7.—Exp. CA 49–6. Initial fatigue series. Pouch tissue 130 minutes after first eugenol application. × 100.

FIGURE 8.—Exp. CA 69–1. Recovery period. Pouch tissue 36½ hours following initial fatigue series. × 100.





Chairman Wangensteen: We will now proceed with the discussion of these papers. In large audiences one gets the impression occasionally that the essayist speaks sometimes to vie for approbation of the audience, like gladiators do for that of the spectators, rather than for the purpose of trying to effect a meeting of the minds. I think that a meeting composed as this one is should, following good techniques of construction, bring out useful information.

There are many members of the National Advisory Cancer Council here. Dr. Ivy is chairman of the Council. I wonder, Dr. Ivy, if you would like to begin the discussion of the papers we have heard so far.

Dr. Ivy: I have only three questions to ask. These questions may be like a lot of questions asked in discussions where the answer is that the experiment hasn't been done yet. I should like to ask Dr. Shacter if any of the cases on his list of cancer of the stomach were of the scirrhous type of cancer of the stomach. I should like to ask Dr. Code if he took the body temperature of his dogs when he assayed for the inhibitory substances, because fever and other manifestations of toxicity are usually associated with an inhibition of acid secretion.

Dr. Hollander has been working on this subject of the irritation of the gastric mucosa with eugenol for a couple of years. I wonder if he has made any observations on the effects of repeated application of eugenol to the gastric mucosa. Has he obtained anything of the nature of atrophic gastritis?

Chairman Wangensteen: I think I shall ask those to whom those questions were directed to keep them in mind, and we will proceed with further discussion. We are fortunate to have amongst surgeons those who include other borders of knowledge. One of our surgeons has been derelict enough to become an enzymologist. Dr. Huggins, will you say something?

Dr. Huggins: The only thing I can say is that I am completely lost in these proceedings. I am thoroughly a layman here. One of the things I find interesting is what a milligram of hydrochloric acid is; I cannot visualize that. Another thing is what a degree of acid is, 60 or 80 degrees. I think if we could get it to the milli equivalent it would make a little more sense to me as a layman.

Dr. Ivy: I might point out to Dr. Huggins that clinical units are numerically equivalent to milli equivalents.

Chairman Wangensteen: We are meeting right here in the midst of an area where there has been a good deal of interest in the general problem. I see in the audience a veteran, although of lively spirit, and I am going to call on Dr. Arthur Bloomfield of the faculty of this University.

Dr. Bloomfield: I feel a good deal like Dr. Huggins; all I can do is ask a question or two. There are some very interesting points in these papers.

I was much interested in Dr. Shacter's paper about lactic acid, because when we were medical students 40 years ago, all the books mentioned lactic acid as one of the characteristics of gastric secretion in cancer. Of course, cancer of the stomach is a very general term. You can have a huge sloughing ulcerating growth in the fundus; you can have a small growth in the pylorus. I wonder if there is any parallelism between the anatomy of the cancer and the output of lactic acid.

I also take issue with him very sharply on his statement that human gastric juice cannot be worked with accurately. That is simply a matter of technique. If the patient has fasted 18 hours before the tube is put down, if he is furnished with a basin to spit into and plenty of gauze and is asked not to swallow any secretions, and if careful continuous aspiration is made, one can usually tell whether there is any duodenal regurgitation because usually, not always, there will be a faint bile tinge. We have so many curves that go in a smooth fashion after histamine that I think there is no question that after continuous aspiration one is getting all the juice and nothing

but the juice. If there is any regurgitation one can recognize it. I don't think it is quite as hopeless as he has made out.

Chairman Wangensteen: These papers are now open to general discussion.

Dr. Homburger: I should like to comment on Dr. Shacter's paper on lactic acid. In the course of a program to reevaluate some of the so-called diagnostic tests for cancer, we have studied exudates of pleural and peritoneal cavities for their lactic acid content.

I think that while Dr. Shacter's studies have shown very well that glycolysis takes place in gastric tumors in man when glucose is given intravenously, our observations show that even without intravenous addition of glucose there is glycolysis in tumors. In exudates the lactic acid values varied, being elevated in the majority of cases where there was cancer present. These observations merely repeated and expanded work found in the literature of the twenties and thirties. In no case of sterile exudate or transudate where no cancer was present has there been found a high lactic acid value. At present these studies extend to about 50 fluids.

We are aware of Robertson's work on the fermentative origin of gastric lactic acid. Nevertheless, we feel that this has to be reinvestigated and are at present studying lactic acid in the fasting stomach, using a modification (R. L. Markus) of the Miller-Muntz method. Of 16 cases with no gastric complaints, only 2 had levels of more than 20 mg. of lactic acid per 100 ml. These were 2 cases of pernicious anemia. Of 8 cases with benign gastric lesions, only one had more than 20 mg. of lactic acid per 100 ml. of gastric juice. Of 9 histologically proven cases with gastric cancer (none of whom had stagnation of gastric contents), only one showed less than 20 mg. of lactic acid per 100 ml. This patient had a lesion of the cardia. When histamine was given, the amount of lactic acid was diluted as gastric secretion increased.

We believe that the lactic acid chapter in this field should be reopened for intensive exploration with modern methods.

Chairman Wangensteen: Any discussion? Any comment or questions?

Dr. Code: I am a neophyte in discussions dealing with cancer, but one of the questions I would like to ask Dr. Hollander is this: I have been impressed by the work of my colleagues in surgical pathology in which they have taken the secretion of an organ, centrifuged it, and observed the cellular components, or they have scraped the surface of an organ, and by one or other of these procedures they have obtained a cross section of the surface cells of the organ. It seems hard to insert into the stomach something that would scrape representative cells off the surface.

Do you think, Dr. Hollander, that eugenol might be used as a means of obtaining a sample of the surface cells of the gastric mucosa? A gastric tube might be passed, some eugenol put down, the secretion then withdrawn, centrifuged, and the cellular deposit examined. The desquamating action of eugenol would be used in this way to obtain a sample of gastric mucosa cells for diagnostic and study purposes. Do you think we would have a representative sample, a spotty sample, or would the material be so toxic that the patient would become sick?

Chairman Wangensteen: Some questions have been asked here. Dr. Shacter.

Dr. Shacter: In answer to Dr. Ivy's question whether any of the gastric carcinoma patients studied had tumors of the scirrhous type, one patient of our series had a scirrhous carcinoma. The rise in gastric lactic acid following glucose administration to this patient was similar to that observed in patients with gastric adenocarcinoma. Apparently there is no relationship between the type of the carcinoma and the amount of lactic acid produced.

Turning now to Dr. Bloomfield's statement regarding control of regurgitation from the intestine, control of regurgitation would, of course, be very desirable. In our investigation, however, where no special precautions were taken, we were sometimes troubled with regurgitation.

Chairman Wangensteen: Dr. Code, another question addressed to you by Dr. Ivy was about temperature.

Dr. Code: I would not want to minimize the reactions which do occur when material

of this nature is given, in fact when material of the nature of any of the mixtures which we have so far used is given intravenously to the dog. There are two types of reactions: (1) An immediate reaction associated with a fall of blood pressure from which the animal promptly recovers. (This reaction does not always occur.), and (2) a later delayed reaction in which there is a rise of temperature. (This also does not always occur.) We have encountered these reactions following injections of the enterogastrone samples we have tested, the urogastrone samples we have used, and the material we have obtained from gastric juice.

At the present, we do not know whether the material producing these reactions also produces the inhibition. The only thing I can say, Dr. Ivy, is that if we take the temperature every 15 minutes and plot the temperature rise against the inhibition produced, we have not generally obtained a correlation.

In some cases we have obtained a rise of temperature and no inhibition of gastric secretion. Sometimes we have observed marked inhibition and no rise in body temperature. These observations have suggested to us that there is something else here deserving further investigation.

As far as the milligrams of HCl are concerned, we are happy to record our results in any way most helpful to those reading or listening to our papers. This varies from audience to audience—milligrams, clinical units, or milli equivalents. I am in complete agreement with Dr. Huggins that the milli equivalent is usually the most satisfactory unit by which to record the output of HCl from gastric pouches.

Chairman Wangensteen: Dr. Hollander, I believe Dr. Ivy addressed a question to you,

Dr. Hollander: I think the question regarding evidence of atrophic gastritis was the first one asked. It is my feeling that we already have indication of early stages in the development of a gastritis with cysts which are lined by typical flat cells. Possibly these are the same as we see on the surface of the mucosa in the early stages of regeneration following eugenol desquamation. We also see tortuous crypts and lymph follicles in significant number.

The problem that disturbs me, however, is this: Since we are tracing through the process of these recovery changes only in their earliest stages, it is necessary to develop a satisfactory method for quantitizing the incidence of these characteristics of gastritis. I need not tell you how difficult such an effort must be, particularly in relation to histological material of this kind. Still, that is the problem which faces us in an attempt to answer Dr. Ivy's question; that, and the need for somebody with the trained eye of a pathologist who can help us interpret these things. Nevertheless, it is my feeling, at this time, that very early stages of gastritis are present.

Regarding Dr. Code's question about the possibility of using eugenol as a chemical-scraping device, not only is that a possibility but we actually have been working with eugenol in humans as well as in dogs with this end in mind. The eugenol serves even more effectively than a mechanical scraper because one can control the extent and duration of the desquamation more effectively with a chemical agent.

The material is toxic, and we have already conducted studies on toxicity in dogs and rodents, so we know what doses and what duration of application are safe for the human. We have run a sizeable series of studies on individuals without gastric malignancy, with evidence of gastritis, with ulcer, and a few with no obvious gastric disturbance. We, therefore, know what the possibilities of the technique are, and we have already done a few such studies on patients with gastric malignancy. One member of our group, Dr. Sonnenblick, was training himself to study and interpret Papanicolaou smears of such clinical material. Unfortunately, he left us about a year ago, and he has not yet been replaced, but such a study is definitely one of the major aspects of our program.

Dr. Ivy: I should like to ask Dr. Hollander if you could not substitute ethyl alcohol for eugenol, because 30-percent ethyl alcohol will do the same thing to the rat, cat, and dog stomach as was indicated on the slides, and ethyl alcohol might be less toxic than eugenol.

Dr. Hollander: In some of the earliest phases of our work you may recall, Dr. Ivy,

we studied a series of mild irritants which were commonly reported in the literature as being mucigogues. One of those was alcohol. When we used alcohol at a concentration of about 75 to 85 percent, we found it exercised essentially the same action as clove oil, water saturated with ether, and a number of other such agents.

I was afraid for the purposes of our study, however, that alcohol would also exercise an acid-stimulating function, if not immediately, then within the space of an hour or so. For that reason I avoided it in seeking a chemical agent which could be adopted as a standard type of mucigogue. In the course of our working with this series of substances, we finally decided to use eugenol, which is the main component of the clove-oil mixture, for this purpose.

Chairman Wangensteen: Dr. Glick, I believe several questions were directed to you. Do you want to defend yourself at this juncture?

Dr. Glick: Concerning the measurement of nitrogen we didn't do any of that. I think it would be a good idea though, and it would be rather well to try to correlate the urease activity with nitrogen content.

We did refer our data only to the unit of volume of the mucosa—that is activity per section, of known diameter and thickness.

Concerning the discrepancy between the acidity measurements, total and free acid, the data were obtained from the patients' chart; tests were run routinely in the laboratory. I, too, was bothered about the great total and free acid difference in some cases. That is the reason I avoided mentioning the total acid. I think there may have been some duodenal regurgitation.

I do think, however, that it is rather striking to get this correlation between the urease and the free acid.

Chairman Wangensteen: Do any of the essayists who have spoken so far have any additional comment? Dr. Hollander?

Dr. Hollander: May I make one or two remarks about some of the comments made?

I would like to add my voice most emphatically to Dr. Huggins'. It seems to me that we should long ago have adopted the common physical-chemical concepts and terminology for describing the chemical composition of the various digestive secretions. It is true, as Dr. Ivy said, that clinical units and degrees of acidity are numerically identical with normal concentration, but I don't consider that a sufficient reason for continuing the use of the former units.

Another point is the one Dr. Bloomfield raised regarding the nitrogen content in gastric juice. Some of you may recall that years ago Dienst in Europe reported a lot of data on the nitrogen content of human gastric juice in relation to the acidity of the specimens. Shortly after I first saw this paper, I did a statistical analysis of the data presented in it.

As I remember, there were over a hundred pairs of these values, and I found that that there was a very high correlation between nitrogen content and acidity, but the correlation was negative. That is, the higher the acidity, the lower was the nitrogen content. Furthermore, I extrapolated the rectilinear relation to the point where it crossed the acidity axis, which corresponds to a nitrogen content of zero. The acidity at this point was about 165 millinormal, which is the value I had previously inferred to be the concentration of HCl in pure, base-free parietal secretion. From this observation it may be concluded that the pure acid secretion is also free of nitrogenous substances in any significant quantity.

Chairman Wangensteen: If none of the essayists wish to speak further, we will go on with the program. The first paper in the second group is entitled Preparation of Emulsions and Suspensions Containing Carcinogenic Hydrocarbons. The essayist is Dr. Egon Lorenz, head of the Biophysics Section of the National Cancer Institute.

PREPARATION OF EMULSIONS AND SUSPENSIONS CONTAINING CARCINOGENIC HYDROCARBONS[1]

By Egon Lorenz, *National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.*

For experiments originally designed to study the fate of carcinogens in the animal body following different routes of administrations, emulsions and suspensions were prepared which ultimately proved of great value in tumor induction in general and especially in tumor induction in the gastrointestinal tract.

The composition and method of preparation of such emulsions and suspensions are given in the following:

Olive-Oil Emulsion

Dissolve 400 mg. methylcholanthrene (M.C.A.) in 100 ml. of warm olive oil.

Add solution to 900 ml. of distilled water containing 8 ml. of 1 N-NaOH.

Shake for at least 12 hours in fast shaker.

Keep pH adjusted to 9 as emulsion creams out below pH 8.

To obtain acid-stable emulsion (down to pH 2), incorporate 0.5-percent Aerosol OT (dioctyl ester of sulfosuccinic acid).

Mineral-Oil Emulsion with Aerosol OT

100 ml. light mineral oil
5 g. cetyl alcohol
6 g. glyceryl monostearate
1 g. cholesterol
400 mg. M.C.A.
5 g. Aerosol OT

Combine above materials at 70–90° C.

Pour into 800 ml. of dilute NaOH solution of pH 9. Shake briefly.

Bring volume to 1 liter with dilute NaOH solution.

Keep pH adjusted to 9.

Mineral-Oil Emulsion with Sulfonated Castor Oil

80 ml. light mineral oil
5 g. cetyl alcohol
5 g. glyceryl monostearate
1 g. cholesterol
400 mg. M.C.A.

Combine above materials at 70–90° C.

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

Let cool, add 50 ml. of sulfonated castor oil (50 percent sulfonated), and mix thoroughly in mortar.

Add slowly under constant stirring approximately 100 ml. of dilute NaOH solution of pH 9 at approximately 40° C. When emulsion has formed bring volume to 1 liter with dilute NaOH.

Keep pH adjusted to 9.

Mineral-Oil Emulsion with Sodium Taurocholate

80 ml. light mineral oil
5 g. cetyl alcohol
5 g. glyceryl monostearate
1 g. cholesterol
400 mg. M.C.A.

Combine above materials at 70–90° C.

Dissolve 5 g. sodium taurocholate in 800 ml. of dilute NaOH solution of pH 9. Add oil solution slowly under constant shaking.

Bring volume to 1 liter with dilute NaOH.

Keep pH adjusted to 9.

Suspension in Horse Serum

Dissolve M.C.A. in excess of ether.

Add M.C.A.-ether solution dropwise to horse serum.

Remove ether by bubbling strong current of N_2 through serum at temperature of about 10° C.

Finally, let warm up to room temperature to remove last traces of ether.

Suspensions of high M.C.A. content may require additional shaking with glass beads to break up agglomerations of M.C.A. particles.

Maximum concentration obtainable approximately 30 mg. per ml.

Although the horse serum suspension is rather easily made, it has the disadvantage of sensitizing the animals when repeated injections are necessary. Therefore the preparation of suspensions with wetting agents and protective colloids was worked out.

Suspension Prepared With Wetting Agent and Protective Colloid

PRECIPITATION METHOD

Basic solution:

0.01-percent Aerosol OT.
0.1-percent methylcellulose (Methocel) 4,000 c. p. s.
pH adjusted to 9 with NaOH.

Dissolve M.C.A. in excess of acetone. Add M.C.A. acetone solution dropwise and boil Aerosol, methocel solution under reduced pressure passing a stream of N_2 through it.

Maximum concentration obtainable approximately 10 mg. of M.C.A. per ml.

Keep pH adjusted to 9.

Suspension Prepared With Wetting Agent and Protective Colloid

COLLOID MILL METHOD

Basic solution:

0.01-percent Aerosol.

0.1-percent Methocel 4,000 c.p.s.

pH adjusted to 9 with NaOH.

Wet M.C.A. crystals with small amount of basic solution by mixing in mortar. Add basic solution.

Pass through colloid mill for about 30 minutes with frequent stops whenever suspension gets too hot. Concentrations up to 30 mg. per ml. obtainable.

Keep pH adjusted to 9.

It might be mentioned here that the last method has been used also for making suspensions of steroids; an aqueous suspension of stilbesterol has been used clinically.

Emulsions as well as suspensions containing methylcholanthrene were given to mice of different strains instead of drinking water with the following chief results so far as the gastrointestinal tract is concerned:

The nonstabilized olive-oil emulsion almost exclusively induces tumors of the small intestine. When this emulsion is stabilized with Aerosol, it will induce tumors of the forestomach as well as tumors of the small intestine.

The mineral-oil emulsions induce mainly tumors of the forestomach; the emulsion containing Aerosol induces more tumors and at an earlier time than the other mineral-oil emulsion.

Suspensions induce tumors of the forestomach as well as tumors of the small intestine. Their carcinogenic action is less pronounced than that of the emulsions.

No tumors and no changes in the glandular stomach were induced in mice either in those strains with normal mucosa or in those with adenomatous hyperplasia of its mucosa (I, dba). The induction of ulcers in the glandular stomach of mice by either acid pepsin solution or interrupted exposure to low pressure (equivalent to an altitude of 25,000 feet) and the simultaneous oral administration of olive-oil emulsion or of a suspension containing methylcholanthrene also failed to induce tumors of the glandular stomach. Likewise, the stomach of guinea pigs proved refractory although these animals were given a methylcholanthrene suspension instead of drinking water for periods up to 2 years. On the other hand, as has been shown, tumors of the glandular stomach can be induced by injecting methylcholanthrene suspensions into the wall of the glandular stomach of mice, hence the gastric glandular mucosa of mice is susceptible to tumor formation.

Therefore, I have come to the conclusion that in carcinogenesis of the glandular stomach of mice and perhaps man also, we should not look too much for an extrinsic factor contained in the food but rather for intrinsic factors.

Chairman Wangensteen: Thank you, Dr. Lorenz, for a very interesting paper. The next paper is titled Adenocarcinoma and other Lesions of the Glandular Stomach of Mice, Following Intramural Injection of 20-Methylcholanthrene. The essayist is Dr. Harold Stewart, head of the Pathology Section, National Cancer Institute.

ADENOCARCINOMA AND OTHER LESIONS OF THE GLANDULAR STOMACH OF MICE, FOLLOWING INTRAMURAL INJECTION OF 20-METHYLCHOLANTHRENE [1]

By HAROLD L. STEWART, W. V. HARE, EGON LORENZ, and J. G. BENNETT, *National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.*

The experimental induction of adenocarcinoma of the glandular stomach in mice was reported by Stewart and Lorenz in 1942.[2] At that time a number of different experiments had been completed in which 20-methylcholanthrene, 1,2,5,6-dibenzanthracene or 3,4-benzpyrene had been introduced into the wall of the stomach in several different ways: dissolved in lard or mineral oil, suspended in horse serum, or impregnated on a cotton thread. A total of 293 mice of several strains were used in this experiment. At autopsy the following tumors of the glandular stomach were found: adenoma (precancerous lesion) 44; adenocarcinoma 8; adenoacanthoma 8; combined neoplastic epithelial lesions and sarcoma 20; and sarcoma 30. All the carcinomas developed in mice receiving an injection of 0.8 mg. of methylcholanthrene in heavy horse serum suspension. Following the preparation of the paper for publication, adenocarcinoma of the glandular stomach was found at autopsy in 3 additional male mice of C3H, I, and C57 brown strains which received, respectively, 0.4 mg. of 20-methylcholanthrene dispersed in horse serum, 0.6 mg. of 20-methylcholanthrene in the same dispersion, and 0.14 mg. of methycholanthrene in mineral oil.

The present experiment was designed to test the effect of a single dose of 20-methylcholanthrene injected intramurally into the glandular stomach upon the induction of various types of gastric tumors in mice of 5 different strains. To date 250 mice of strains C, C57 black, C3H, C3Hb, and dba of which 124 were males and 126 females, have been autopsied.

The operative procedure was carried out under nembutal anesthesia administered intraperitoneally. The abdomen was shaved and washed with 70-percent ethyl alcohol and the stomach delivered through an epigastric midline incision. By means of a 1-cc. tuberculin syringe fitted with 27-gage needle, 0.01 cc. of a suspension containing 0.3 mg. of 20-methylcholanthrene was injected into the anterior wall of the pyloric portion of the glandular stomach. An attempt was made to inject the material into the submucosa. The composition of the suspension is as follows: distilled water; methylcholanthrene, 30 mg. per cubic centimeter; Aerosol, O. T., 0.01 percent; Methocel (4,000), 0.1 percent; ethyl alcohol, 1.0 percent; NAOH to pH 8.0. The peritoneum and skin were closed with

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] STEWART, H. L., and LORENZ, E.: Adenocarcinomas of the pyloric stomach and other gastric neoplasms in mice induced with carcinogenic hydrocarbons. J. Nat. Cancer Inst. 3: 175–189 (1942).

silk sutures, and the animal was returned to its cage. The animals were 2 to 5½ months old at the beginning of the experiment. They were given Purina Dog Chow and water ad libitum. When palpable tumors seemed to be large enough to portend imminent death or when the animals appeared ill, they were etherized and autopsied immediately. In those found dead, the possible presence and degree of post mortem autolysis were considered in the evaluation of the histologic changes.

The stomach was the point of first attention in all autopsies. In some cases it was opened immediately and spread out on a stretching board, in others, it was injected in situ with fixative after tying off the esophagus and duodenum. Freshly prepared Zenker's acetic solution was used as the fixative in all cases. All histologic preparations were stained with hematoxylin and eosin and with McManus' periodic acid stain. From time to time various other special stains were used, including Van Gieson's connective tissue stain, various silver impregnation methods for reticulum, and phosphotungstic acid-hematoxylin.

The term "carcinoma" has been used only for those epithelial neoplasms that extended through all muscle layers onto the serosa. The term "precancer" has been used in a broad sense, including early lesions with only atypical glands in the mucosa as well as those advanced lesions in which the neoplastic glands have invaded the lamina propria, muscularis mucosae, and muscularis propria, but which have not penetrated the peritoneum.

Of the 250 mice, 90 developed precancerous lesions of the glandular stomach, 8 adenocarcinoma, 2 adenoacanthoma, 37 mixed neoplastic epithelial lesions and sarcoma, and 24 sarcoma. There were 2 mice which developed squamous cell carcinoma of the forestomach. Mice of strain dba, although they develop an adenomatous hyperplastic gastritis of the glandular stomach spontaneously, were resistant to the induction of precancerous lesions, adenocarcinoma, and sarcoma following injection of methylcholanthrene into the wall of the glandular stomach. The mice of the other 4 strains were approximately equally susceptible to the induction of epithelial neoplasms. There was no sex difference.

Although the mice of the C57 black strain were as susceptible to the induction of epithelial tumors as those of the other three strains, they were more resistant to the induction of local sarcoma. Since the development of sarcoma is an undesirable feature in experiments of this type designed to study the problem of gastric carcinoma, mice of the C57 black strain may prove to be the best test object for such work.

The precancerous lesions when calculated on a monthly basis were fairly evenly distributed throughout a period of 1 to 18 months following operation. With a single exception, all the malignant tumors occurred between 2 and 11 months. This exception was an adenocarcinoma which appeared at 18 months.

Chairman Wangensteen: Thank you, Dr. Stewart, for bringing us this convincing evidence of experimental production of cancer in the stomachs of mice. The next paper is The Occurrence of Gastric Lesions in the Rat as a Result of Feeding Tributyrin. It is to be read by one of the authors, Dr. D. H. Copeland, Alabama Polytechnic Institute.

THE OCCURRENCE OF GASTRIC LESIONS IN THE RAT AS A RESULT OF FEEDING TRIBUTYRIN [1] [2]

By W. D. SALMON and D. H. COPELAND, *Laboratory of Animal Nutrition, Agricultural Experiment Station of the Alabama Polytechnic Institute, Auburn, Ala.*

The development of a severe hyperplasia of the forestomach in rats receiving diets containing tributyrin was observed in this laboratory in 1934 (*1*). More recently this condition was again produced in order to make a thorough study of the lesions. The results are reported in this paper.

EXPERIMENTAL PROCEDURES

Rats of the AES strain weighing 50–85 g. were placed in individual cages with wire floors. They were fed and watered daily and weighed weekly. It was found that rats of this weight tolerated the diets better than lighter rats. The composition of the diets used is given in table 1. Occasionally a few rats would refuse the experimental diet. In such cases they were given a complete synthetic diet without a butyric acid compound for one or more days.

TABLE 1.—*Composition of diets*

Ingredients [1]	Butyrin 2A	Butyrin 2B	Butyric acid	Ethyl butyrate [3]
	Percent	*Percent*	*Percent*	*Percent*
Casein [3]	18	18	18	18
Salts No. 5 [3]	4	4	4	4
Sucrose	52. 8	52. 8	52. 8	52. 8
Tributyrin [4]	25	15		
Butyric acid [4]			25	
Ethyl butyrate [4]				25
Lard		10		
Choline	. 2	. 2	. 2	. 2

[1] Vitamins in mg./kg. of diet: Thiamine 2, pyridoxine 2, riboflavin 4, Ca pantothenate 10, niacin 20, inositol 200, carotene 4, a-tocopherol 50, calciferol 0.125.
[3] J. Nutrition 33: 155–168 (1947).
[3] Butyl acetate and butyl butyrate were also tested in the same diet as used for ethyl butyrate.
[4] The tributyrin and other esters and the butyric acid were obtained from Eastman Kodak Co.

A considerable number of rats died during the first week, apparently of starvation. Rats surviving longer than this were necropsied after experimental periods varying from 3 to 35 weeks. The rats were killed

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] Published with the approval of the Director of Alabama Agricultural Experiment Station. This work was supported in part by grants from the Nutrition Foundation, Inc., and from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council. Part of this material was presented at the Atlantic City meeting of American Association for Cancer Research, April, 1948.

by carotid bleeding. The stomachs were removed, cut along the lesser curvature, and washed out with normal saline. They were pinned out on a cork and fixed in Bouin's or Zenker-formol. The fixed tissues were then cut into strips so that sections extending through both the forestomach and glandular stomach could be obtained. Paraffin sections were prepared and stained by hematoxylin-eosin, Massons trichrome, or Mallory's triple stain.

RESULTS

Survival and Growth

One hundred thirteen rats were started on experiment. Thirty died during the first week. In some of the 30 early-succumbing rats, the stomachs showed ulcers or small discrete papillomas. However, these lesions were very mild as compared with those in the stomachs of rats receiving tributyrin diets for longer periods of time. These 30 cases will not receive further consideration in this report. Eighty-three rats came to necropsy after receiving the experimental diets for 3 to 35 weeks.

Rats receiving the tributyrin diets averaged about 10 to 12 g. gain in weight per week up to 6 weeks, which is about one-third of the normal rate of gain for rats of this strain. Rats on the ethyl butyrate diet averaged about 18 g. gain per week. The appearance of these rats showed that the ethyl butyrate was tolerated much better than the tributyrin. Surprisingly, both butyl acetate and butyl butyrate were also tolerated better than the tributyrin. Butyric acid, however, was poorly tolerated, the rats barely maintaining their weight on this diet.

Pathology [1]

Sixty-six rats receiving tributyrin diets were necropsied. Grossly the stomachs were greatly enlarged and exhibited numerous irregular protuberances on the external surface. In some cases well-circumscribed, white nodules projected from the outer surface of the forestomach. In five rats similar nodules were visible on the external surface of the glandular portion of the stomach. Marked hyperplasia of the epithelial lining and large papillomatous growths were grossly evident in the forestomach of all rats. There were occasional ulcerated areas and craters opening to the inner surface (pl. 58, fig. 1). Gross evidence of involvement of the glandular stomach was relatively infrequent.

Microscopic examination of the gastric tissues revealed hyperplasia, hyperkeratosis, papillomatosis, acanthosis, infiltration, and occasional ulceration. The entire mucosa of the forestomach of the tributyrin rats was a mass of papillomas, causing a tremendous thickening of the wall. (Compare *A* and *B*, fig. 2, pl. 59.) The papillomas were composed of proliferations of hyperplastic epithelium thrown into convoluted folds separated only by connective tissue. These proliferations were covered with keratin in concentric whorls and in a flamelike fibrillary arrange-

[1] The authors express their appreciation to Dr. H. L. Stewart of the National Cancer Institute for his assistance in the interpretations relating to some of the histological material.

ment. Numerous cysts were found penetrating the muscularis mucosa (fig. 2, *C*). These cysts were lined with stratified squamous epithelium and contained keratin in a lamellated fibrillary arrangement. These growths pushed out the external muscle layers to form a nodule that was grossly visible on the outside surface of the stomach. In numerous cases, spurs of hyperplastic epithelium extended downward from the papillomatous growths and penetrated the muscularis mucosa. Some of these were found deeply placed in the submucosa. In these areas mitotic figures were numerous, indicating a rapid growth of this lesion (pl. 60. fig. 3, *A*, *B*, C).

Lesions in the glandular stomach were encountered in relatively few of the rats. However, some very interesting changes were observed in the glandular stomach of 10 rats receiving tributyrin diets and of 1 rat receiving the butyric acid diet. In 4 of these animals, there were simple cysts lined with columnar epithelium and located in the submucosa. In another animal a peculiar type of cyst, lined by stratified squamous epithelium and containing keratin, was observed in the glandular stomach. It was deeply placed in the submucosa and there was extensive proliferation of the basal cells as shown in plate 61, figure 4, *A*. Serial sections showed no connection between this cyst and the forestomach. In the remaining 5 animals, the lesions consisted of proliferating gland elements which had penetrated the submucosa and infiltrated the external muscle layers extensively. In 2 of the 5 cases, these lesions were located in the middle portions of the glandular stomach and were associated with diverticulosis (fig. 4, *B*). In 3 cases, they were not associated with diverticulosis and were located in the pyloric part of the stomach. In these 3 cases, the muscularis mucosa was penetrated by gland elements deeply placed in the submucosa and muscularis externa. In some areas the elements remained as simple acini or became cystic; in other areas they had proliferated more extensively to assume bizarre forms as shown in figure 4, *C*.

The 4 rats that received the butyric acid diet exhibited papillomatosis and hyperkeratosis but in a somewhat milder form than was observed in the rats that received tributyrin. One of these rats, however, had a glandular lesion and also a cyst near the junction of the forestomach and glandular stomach. Of the 7 rats that received the ethyl butyrate diet, 3 developed mild hyperplasia; the stomachs of the other 4 appeared normal. Only 3 rats were fed the butyl butyrate diet. They developed mild hyperplasia and very mild scattered papilloma. The stomachs of the 3 rats that received butyl acetate were normal.

DISCUSSION

The production of gastric lesions associated with incomplete diets has been extensively studied in the rat, and good bibliographies on the subject are available (*1–7*). The consensus of opinion at present seems to be that epithelial hyperplasia of the forestomach, papilloma, and ulcers tend to be associated with diets of low nutritive value. Satisfactory proof that

any specific dietary deficiency is the primary cause of such abnormalities is lacking.

The possible effects of partial inanition in the rats that received tributyrin cannot be ignored. The diet was frequently refused by some of the animals at the beginning of the experiment, and food intake and weight gain were far below normal throughout the experiment. It is believed that the effects of inanition have been minimized by the use of rats weighing 50 to 85 g. at the beginning of the experiment and by rejecting the rats that died during the first week of the experiment. Moreover, the lesions were less severe in the rats that received the butyric acid diet than in those that received the tributyrin diets for comparable periods of time. Since the rats ate less of the butyric acid diet than of the tributyrin diets, this argues against inanition being the primary cause of the lesions.

Certain harmful effects of tributyrin have previously been reported. Eckstein (*8*) found that diets containing as little as 5 percent of tributyrin were invariably refused by rats. He attributed this to the intense bitterness of the compound. Davis (*9*) administered tributyrin to chickens by pipette in amounts equivalent to about 18 percent of the feed consumed. He reported toxic effects and the production of lesions in the proventriculus and the gizzard. The intestinal walls were inflamed and, in some cases, the mucosa in the fore part of the small intestine was destroyed. In digestion trials with hens, the digestibility of tributyrin was about 25 percent higher in the first trial than in the second trial with the same hen. This was thought to be a result of damage to the digestive tract. In both Eckstein's and Davis' work the tributyrin fed was not stored in the depot fats.

The evidence strongly suggests that the damage to the gastric tissues of the rats in our experiments was a result of a direct irritative effect of the butyric acid preparations. Of these, tributyrin appeared to produce the greatest damage. The lesions produced by the tributyrin diets were much more severe than were produced in this laboratory [4] by diets of the Pappenheimer type (*10*).

At present, these results are principally of academic interest. It is not believed that significant amounts of tributyrin occur in any natural food. The glycerides in butter probably contain more butyric acid than any other food. Complete diets containing 60 percent of butter have been fed to rats in this laboratory and have not produced any apparent injury. Nevertheless, the severe pathological effect of tributyrin on the gastric epithelium is an extremely interesting phenomenon and deserves further study.

SUMMARY

Marked hyperplasia of the epithelial lining and large papillomatous growths were grossly evident on the inside of the forestomach of all rats receiving diets containing 15 to 25 percent of tributyrin for 3 to 35 weeks.

[4] SALMON, W. D., and COPELAND, D. H.; Unpublished data (1947).

Microscopically the following changes were observed: Hyperplasia, hyperkeratosis, papillomatosis, acanthosis, infiltration, and occasional ulceration.

Lesions were observed in the glandular stomach of a few of the rats. These lesions consisted of cysts lined with glandular epithelium or of proliferating gland elements deeply placed in the submucosa and in the external muscle layer.

Tributyrin produced more damage to the gastric epithelium than ethyl butyrate, butyl butyrate, or butyric acid. Butyl acetate did not produce any damage.

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Chairman Wangensteen: Thank you, Dr. Copeland. The next paper is Gastric Cancer Experimentally Induced in Insects by Nerve Severance. The essayist is Dr. Berta Scharrer, Instructor in Anatomy, University of Colorado.

PLATE 58

FIGURE 1.—Gross appearance of stomach of rat receiving tributyrin diet for 9 weeks. The entire forestomach is a mass of papillomas. Note the crater near the junction of the glandular and nonglandular stomach.

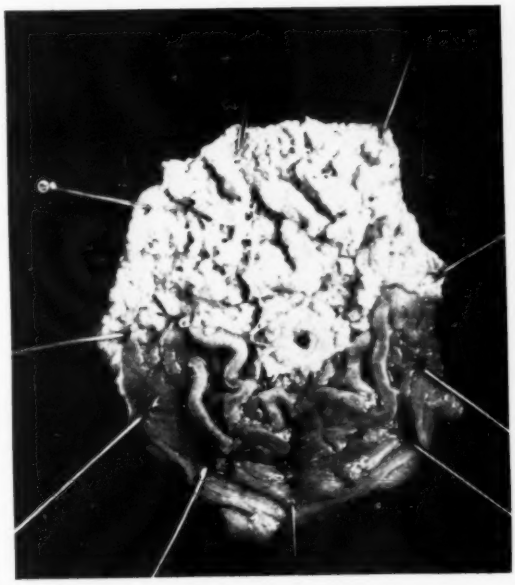
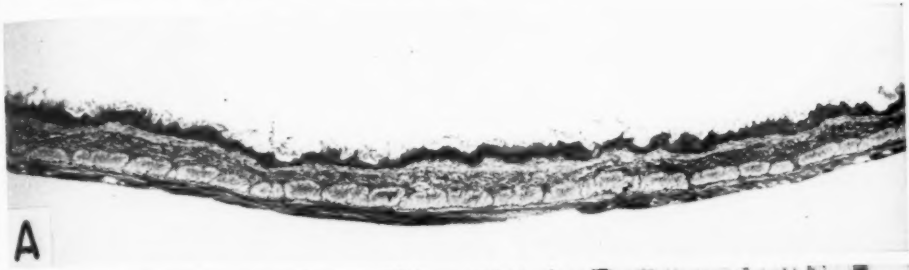
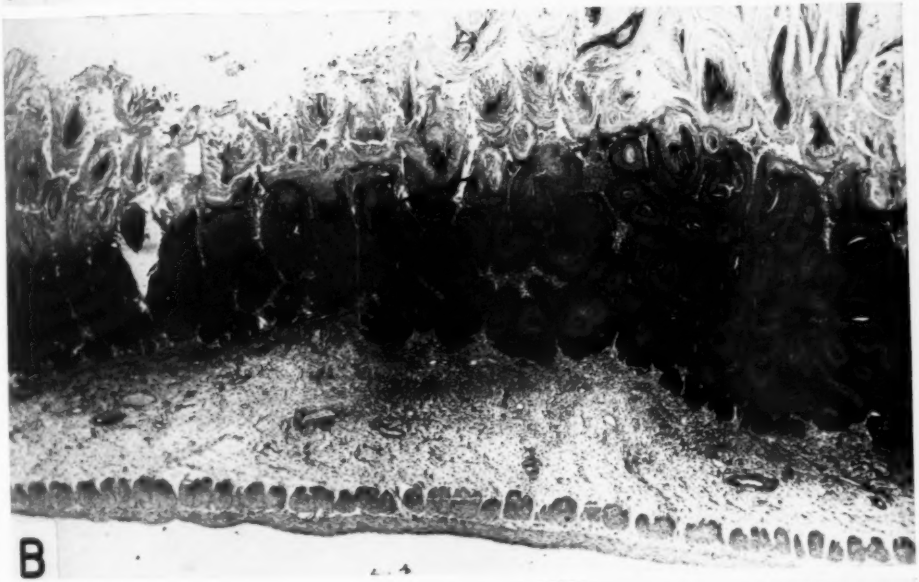


PLATE 59

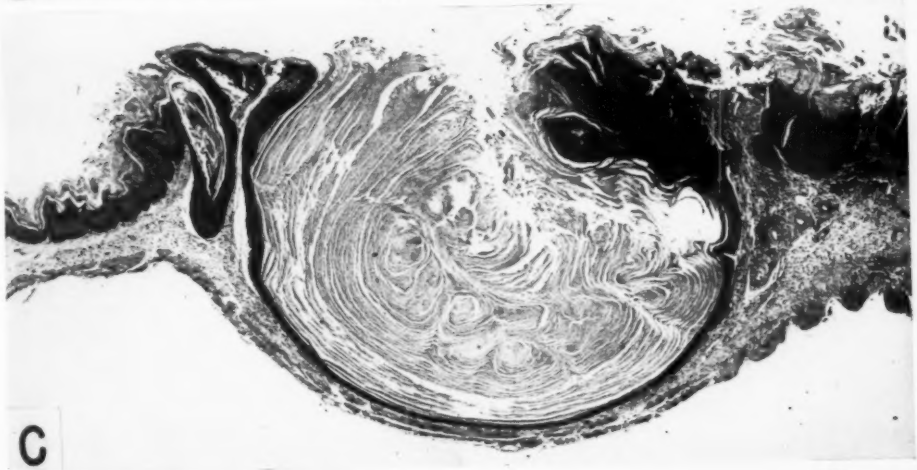
FIGURE 2.—*A*, Section of normal forestomach of rat showing relative thickness of layers. Hematoxylin-eosin stain. × 23. *B*, Section through papilloma to show proliferation of stratified squamous epithelium, hyperkeratosis, and disruption of muscularis mucosa. Hematoxylin-eosin stain. × 23. *C*, Section through crater in figure 1 showing nodular downgrowth into submucosa; this cystic growth is lined with stratified squamous epithelium and contains keratin arranged in fibrillary lamellae. Hematoxylin-eosin. × 23.



A



B



C

PLATE 60

FIGURE 3.—*A*, Area of forestomach showing extensive papillomatosis and hyperkeratosis. Hematoxylin-eosin stain. × 58. *B*, Area of forestomach showing massive downgrowth of stratified squamous epithelium into submucosa with disruption of the muscularis mucosa of the forestomach. Hematoxylin-eosin stain. × 58. *C*, Higher magnification of area of forestomach to show cell structure of hyperplastic downgrowths. Note the extensive proliferation of basal cells of the epithelium; some of these have penetrated the muscularis mucosa. Hematoxylin-eosin stain. × 260.

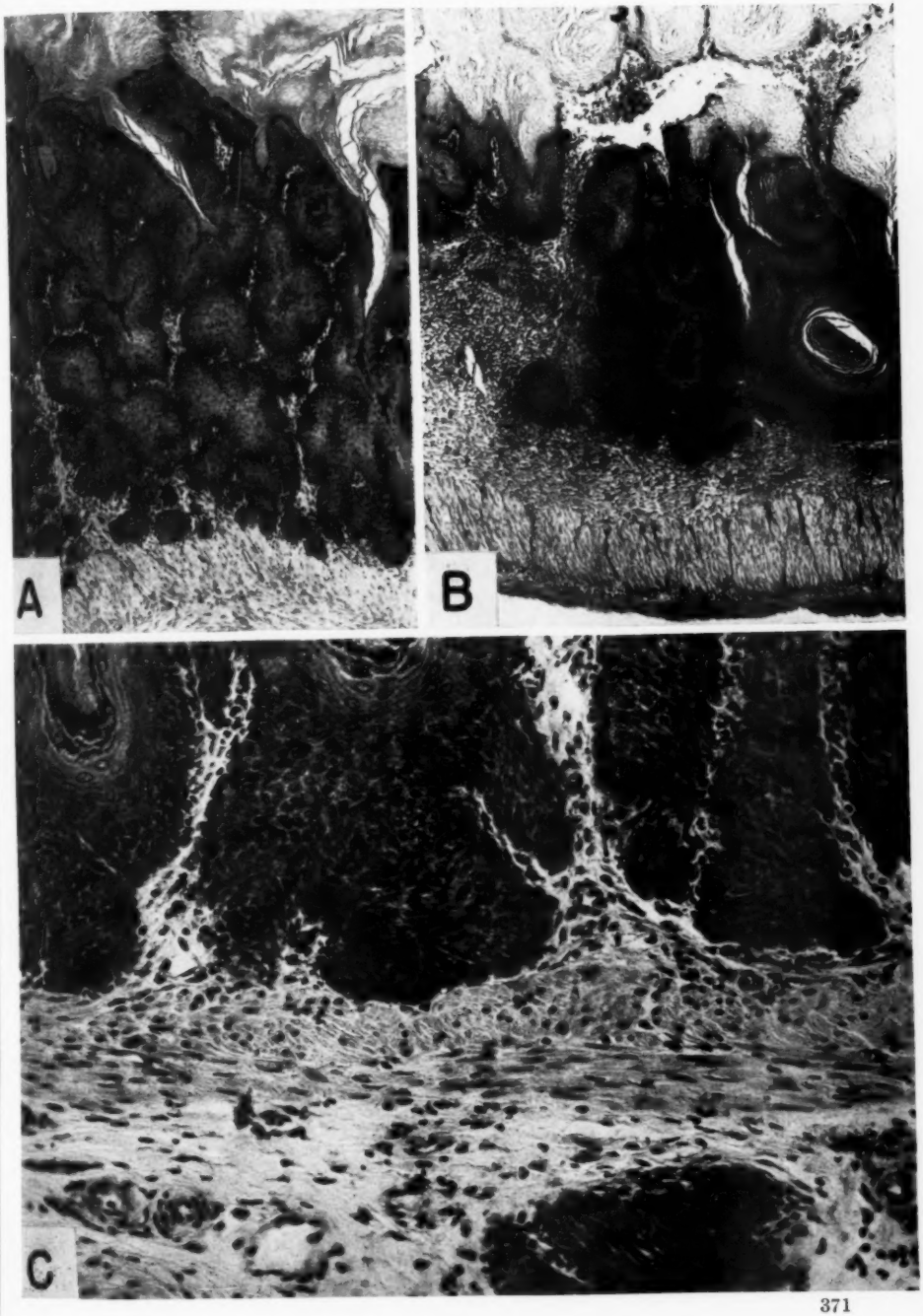
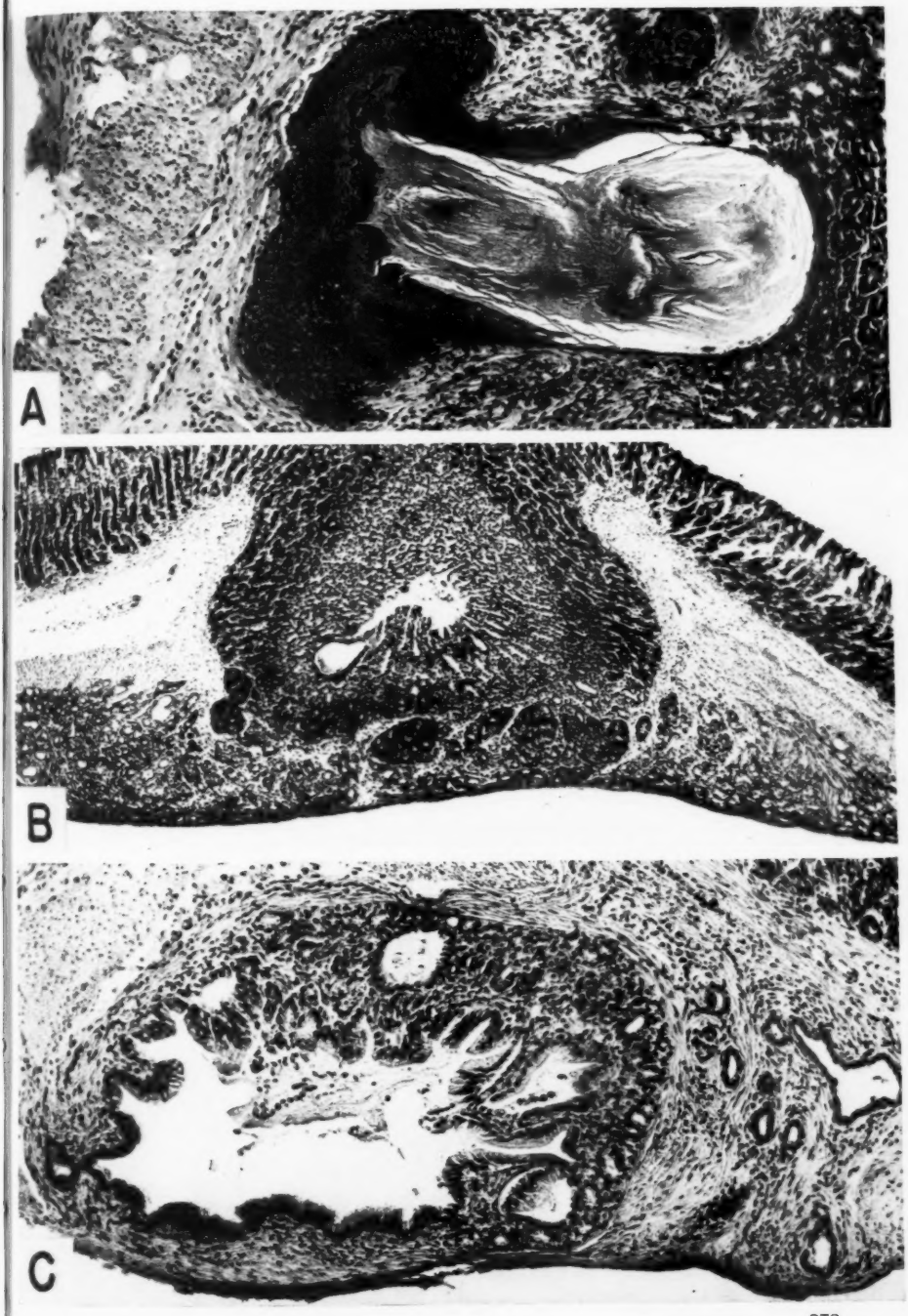

A
B
C

PLATE 61

FIGURE 4.—*A*, Lesions in glandular portion of stomach, lined with stratified squamous epithelium and containing keratin. Note the proliferation of basal cells extending into the submucosa. Hematoxylin-eosin stain. × 110. *B*, Lesion in glandular stomach associated with diverticulosis with extensive glandular proliferation and hyperplasia, extending into muscularis externa. Hematoxylin-eosin stain. × 55. *C*, Lesion in glandular stomach not associated with diverticulosis. Note the proliferating gland elements, deeply placed in the submucosa and muscularis externa. Hematoxylin-eosin stain. × 110.


A
B
C

GASTRIC CANCER EXPERIMENTALLY INDUCED IN INSECTS BY NERVE SEVERANCE [1]

By BERTA SCHARRER, *University of Colorado Medical Center, Denver, Colo.*

Among the various causes and contributory factors that may play a role in cancerous growth, the nervous system has been given little attention in the past. Yet a possible relationship between this important regulatory mechanism and tumor development should not be overlooked. In the few instances in the literature where this problem has been discussed, especially with respect to gastric cancer, it was done on a more or less theoretical basis, unsupported by experimental evidence.

In recent years new light has been thrown on this important question in the course of work in the field of insect physiology originally intended for other purposes.[2]

To state the observed facts briefly, it was found that in certain insects gastric cancer can be induced experimentally by the severance of a nerve, the so-called recurrent nerve, which innervates the stomach. Except for minor details the digestive organs of an insect are structurally comparable to those of higher organisms, the similarity of structure reflecting the similarity of function.

The insect used in this work is a large tropical species (*Leucophaea maderae*) which is related to the roaches. It is eminently suited for experimentation on account of its size, its breeding habits, and its general hardiness. The recurrent nerve which belongs to the autonomic system supplies the anterior portion of the digestive tract as well as the salivary organs (which likewise develop tumors after nerve severance).

The cutting of the nerve was performed at three different levels, the results being essentially the same after each type of operation. The most severe tumors and the most frequently observed, after the nerve was severed, were found in the stomach (midgut). They are characterized by their rapid growth, invasive properties, and abnormal cell structure (giant nuclei, pycnosis, necrosis, scarcity of mitotic figures, lack of chromatin). Some of these features are indicative of malignancy.

Experimental induction of these insect tumors is possible in both sexes and in various age groups (nymphs, young, and old adults) although not with the same frequency. Possible relationships between endocrine factors and tumor development are now under investigation. In general, tumor incidence is high after nerve severance (about 75 to 80 percent).

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[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] SCHARRER, B.: Experimental tumors after nerve section in an insect. Proc. Soc. Exper. Biol. & Med. 60: 184–189 (1945).

The tumors of the stomach can be transplanted into normal specimens of the same species.

Spontaneous tumors of the insect stomach, comparable to those experimentally induced, are found in rare instances. An extensive search is being conducted for spontaneous tumors in large numbers of normal specimens. The few cases detected so far are considerably less severe than those following nerve injury.

In summary, it can be stated that in an insect (Leucophaea) interference with the innervation of the stomach causes cancerous growth. To date, similar results have not been obtained in higher organisms. However, the observations in insects should be of sufficient interest to stimulate a renewed search for possible nervous factors in human gastric cancer.

Chairman Wangensteen: Thank you, Dr. Scharrer, for bringing us these results from your interesting experiment. The last paper of the morning is The Role of Mucous Cells in the Production of Gastric Neoplasms. The essayist is Dr. Edward L. Howes, Associate Clinical Professor of Surgery, Columbia University.

THE ROLE OF MUCOUS CELLS IN THE PRODUCTION OF GASTRIC NEOPLASMS [1] [2]

By EDWARD L. HOWES, *Department of Surgery and Cancer Research, Columbia University, New York*

In a recent publication, experimental evidence was presented that mucous or mucous stem cells give origin to new growths of the stomach (*1*). It was shown that when a thread containing a carcinogen was buried in the wall of the stomach, an ulceration occurred in the mucosa over it. Then an epithelial-lined sinus or sulcus formed in the area, depending on how much of the thread sloughed. The epithelium originated from basophilic cells of the surrounding uninjured mucosa that promptly moved down into the defect or along a retained portion of the suture and proliferated to line and form a sulcus or sinus. This basophilic epithelium that now existed below the muscularis mucosa and under different environmental conditions, proliferated farther to form an adenomata or adenocarcinoma. An infiltrating growth resulted when the connective tissue barrier beyond a sinus was destroyed by the carcinogen, while an adenomata formed when the connective tissue barrier about the sulcus remained intact and the epithelial mass extended inward toward the lumen of the stomach.

Similarly, basophilic cells proliferated to line sinuses and sulci formed about threads containing a noncarcinogenic anthracene compound, 1,2-benzanthracene, but the cells did not continue to proliferate as they did in the presence of the carcinogen. Instead they differentiated into layers. Such reorganized epithelium has been observed for a year, and neither acidophilic nor pepsin-secreting cells ever reappeared while the constituent cells continued to give a weak staining reaction for mucus. Lastly, similar basophilic epithelium also came to inhabit the occasional sinus formed about an untreated silk thread that perforated the mucosa. Thus, because basophilic epithelium formed under all three circumstances, injury alone was believed to be responsible for its formation, and, in consequence, the hypothesis has been made that whenever intestinalized epithelium is found in areas where combined epithelium (acid, pepsin, and mucous-secreting cells) ordinarily exists, injury must have occurred. Finally, this study demonstrated that various cells of the combined mucosa had different capacities to withstand injury, that acid- and pepsin-secreting cells were more susceptible to injury than the basophilic cells and that injury might be used experimentally to destroy a

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University, San Francisco, December 13, 1948.

[2] This investigation was supported in part by a grant from the National Cancer Institute, Public Health Service.

sufficient amount of acid- and pepsin-secreting cells to produce large areas of intestinalization and a relative hypochlorhydria or achlorhydria. There is the possibility, moreover, that intestinalized stomach is more susceptible to the action of carcinogens taken orally than the normal stomach which is remarkably resistant to their action. Finally, the behavior pattern toward injury of the normally occurring basophilic cells of the prepyloric portion of the stomach is not known. Consequently, methods have been studied to try to convert large areas of combined mucosa to the basophilic type.

In the first experiments, full depth mechanical destruction was tried. The injured areas healed with basophilic epithelium not as thick as but otherwise indistinguishable by staining reactions from the epithelium lining the pyloric end of the stomach. On the other hand, hypochlorhydria could not be produced in this way, for the animals died when the necessary amount of combined mucosa was destroyed. Accordingly, other methods were sought. Next, dietary deficiencies were produced that resulted in small ulcerations and hemorrhagic areas in the combined mucosa of the stomach of rats, hoping that when these healed an extensive basophilic transformation and hypochlorhydria would result. Apparently, these lesions were too superficial or too limited in extent, because neither intestinalization nor a diminution in acid secretion followed. Inclusion of excessive amounts of roughage or alcohol in the diet or of hydrochloric or lactic acids in the drinking water did not produce mucous transformation. Repeated injections of histamine, made to cause an irregular secretion of acid, did not produce it nor interference with the blood supply alone. On the contrary, injection of sclerotic substances into the wall of the stomach produced basophilic transformation in a contiguous area, but again the method could only be used to convert a small area of combined mucosa.

Finally, however, a method was discovered that could be used to produce mucous transformation in large areas of combined mucosa. By creating ischemia when free acid was present in the lumen of the stomach, extensive mucous transformation was produced. To create the ischemia, the artery and vein descending on the esophagus and spreading out over the posterior and anterior walls of the stomach from the lesser curvature were isolated and ligated. The vagus nerve was not included in the ligature. Next, the vessels ascending from the pancreas and traveling along the greater curvature were ligated opposite the pylorus. Hydrochloric acid N/10, 1 cc., was then placed in the lumen of the stomach, injected by means of a fine needle inserted through the squamous portion of the organ, or it was inserted by means of an ureteral catheter, passed down the esophagus.

Changes Caused in the Mucosa of the Stomach as the Result of Ischemia Only

Although most of the mucosa remained normal, the changes that did occur were most easily discernible after 24 hours. These changes consisted

in an occasional area of small hemorrhage or small ulcerations in the combined mucosa (Pl. 62, fig. 1, and pl. 63, fig. 3). No changes occurred in the pyloric mucosa (Pl. 62, fig. 2). Microscopically, chief and parietal cells and some superficial mucosal cells were destroyed in the combined mucosa, but destruction extended down only to a layer of basophilic cells situated just above the muscularis mucosa. The submucosa showed a great deal of edema. Occasionally, very circumscribed areas of destruction extended to the muscularis mucosa. Microscopically, the cells of the pyloric mucosa were spared although the submucosa under it showed a great deal of edema (fig. 2).

The edema disappeared after 48 hours. Within 3 days the destroyed areas were completely healed, and the morphology of the combined mucosa appeared unchanged.

Changes Produced in the Mucosa With Ischemia Plus Hydrochloric Acid (pH 1.2)

Again the most acute changes were observed after 24 hours. Grossly, these changes consisted of extensive hemorrhages and ulcerations and large plaques of necrosis in the combined mucosa only. Microscopically, there was complete and extensive destruction of all cells down to the muscularis mucosa, including the deep basophilic cells (Pl. 63, fig. 4). Areas of basophilic epithelium along the lesser curvature as well as isolated islands of basophilic epithelium on the anterior or posterior walls were unaffected, however. These were found fully preserved in the middle of areas of destroyed combined mucosa. Again the pyloric epithelium was unharmed, although there was edema beneath.

Whether the areas of basophilic cells along the lesser curvature represented a normal distribution of these cells or resulted from previous injury cannot be stated. The hypothesis can also be advanced that the pyloric mucosa was not affected because ischemia was not sufficiently severe nor prolonged at this end of the stomach. Yet this would not explain why the islands of basophilic cells along the lesser curvature and the occasional ones on the anterior posterior walls survived, and, certainly, the presence of edema and the infiltration of leucocytes beneath the pyloric mucosa suggested that this end of the stomach was severely damaged. The other possibility is, of course, that basophilic cells are in general better able to survive injury, and the evidence both in the previous investigation (*1*), this one, and the one about to be described on transplantation of combined mucosa support the contention that both the basophilic cells of the combined mucosa and of the pyloric mucosa are very resistant to injury.

Healing of the ulcerated and necrotic areas took about 7 days. As it progressed, the stomach shrank in size, and the serosal surface became adherent to surrounding viscera in some areas. An extensive change had occurred, and only small islands of normal mucosa remained. The regenerated mucosa appeared flatter than the normal. Microscopically, the regenerated mucosa was less thick than normal and was composed

entirely of basophilic cells (Pl. 64, fig. 5). Occasionally areas were found where a few chief and parietal cells persisted. In other words, the combined mucosa had undergone mucous transformation.

One month after mucous transformation was produced, injection of histamine disclosed a relative hypochlorhydria (pH 4), and in a few animals an achlorhydria was found. One year later, however, pH readings of 1.5–2.5 were found after injections of histamine. When these stomachs were examined, the mucous transformation was still extensive, but the small residual areas of combined mucosa were hypertrophied.

Effect of Other Acids

By inserting acid of various strengths into stomachs with ligated vessels, the capacity of hydrochloric acid to destroy combined mucosa in the presence of ischemia was found to be in direct proportion to pH. This capacity was least at pH 3.5, the level at which the acid left the stomach. Other acids had this same capacity to destroy combined mucosa, depending on pH, although citric and lactic acids at equivalent pHs seemed to be more destructive than hydrochloric acid. Both orange and grapefruit juice destroyed combined mucosa in the presence of ischemia. The presence of food in the stomach always prevented destruction in spite of the pH of the acid into the lumen. The combined mucosa was also destroyed in the presence of ischemia by causing a secretion of acid by the injection of histamine or by the presence of alcohol in the lumen of the stomach.

Three stomachs with large areas of mucous transformation were subjected to secondary ligation of dilated collateral vessels, and acid was placed in the lumen. The regenerated basophilic mucosa was not destroyed again, suggesting that either transformed epithelium was better able to withstand secondary ligation or that an ischemia, neither sufficiently severe nor prolonged was produced. To support the latter idea, it should be noted that the ligations could not be done as definitively as originally.

A large number of animals have now had ligations of vessels of the stomach in the presence of acid, and they are being maintained on different diets—on excessive amounts of roughage, on cooked oils, and on alcohol, to determine whether new growths will be produced in the modified epithelium. A control group is also being kept for a long period of time to study the spontaneous evolution of the changed mucosa.

Transplantation of Gastric Mucosa

The growth characteristics of the various cells of the mucosa, particularly their relationship to their nutrition and injury, have been investigated by transplanting different portions of the mucosa to a subcutaneous location in the same animal. This work is still in progress but is sufficiently advanced to be worth reporting. As indicated previously, again the mucous cell is the one that survives and proliferates.

In 1942, Kammeraad (*2*) showed that embryonal mucosa of the stomach

of the rat could be transplanted to the anterior chamber of the eye. The mucous cells grew while the pepsin- and acid-secreting cells died. All attempts to transplant the mucosa of the adult rat failed, the cells remaining alive for approximately 6 days only. In 1947, Smith (*3*) transplanted subcutaneously embryonal mucosa and methylcholanthrene in olive oil into homologous strains of mice. The glandular epithelium developed into cysts, but the cells quickly underwent metaplasia to form transitional and stratified epithelium that developed neoplastic tendencies. Embryonal mucosa transplanted without the carcinogen simply formed cysts. Transplants of adult mucosa did not grow. In 1947, Greene [3] also transplanted mucosa of embryonal rats elsewhere in the body with a small crystal of methylcholanthrene placed directly against the mucosa. He obtained disordered growths of cells that morphologically appeared neoplastic. Loeb, in his treatise on transplantation of tissues, did not report successful transplantation of stomach mucosa in the adult animal (*4*).

Because the previous investigators found that transplants of stomach mucosa in adult animals failed to survive more than 6 days, 6 days or longer was selected as suitable time to ascertain whether a successful transplantation had been achieved. After more experience with the growth of the transplants, 14 days was chosen as the ideal time. The transplantation was done in the following manner: A small ellipse of mucosa was made to bulge through an incision made in the serosa and muscular coats. It was then excised with scissors and was kept in the incubator in saline while the wounds in the stomach and abdominal wall were repaired. A small incision was then made over the lower ribs in the axillary line down to the ribs, and the transplant was spread out flat with the mucosal side up. Both adult Wistar and Sherman rats have been used.

The autotransplants, made of both combined and pyloric mucosa, were unsuccessful. However, successful transplantations of combined mucosa were obtained in adult animals by scarification of the site 4 days before the transplantation was made (table 1). This procedure placed the transplant in a bed of granulations rather than at a site where the blood supply had to be proliferated to form a nourishing bed of fine capillaries.

Likewise, combined mucosa was successfully transplanted in adult rats by first incubating it for 10 minutes in either a solution of trypsin or lysozyme (table 1) (Pl. 64, fig. 6, and pl. 65, fig. 7). As reported by Kammeraad (*2*), the mucous cells were the ones that grew while the pepsin- and acid-secreting cells died. The transplanted cells gave a positive stain for mucus and a negative stain for pepsin. Neither methylcholanthrene placed on the serosa or on the mucosa aided the transplant to take, but methylcholanthrene placed in the vicinity of the transplant definitely increased the number of takes (Pl. 66, fig. 9). Also, the noncarcinogenic 1,2-benzanthracene, placed in the area, aided the transplant to take (Pl. 65, fig. 8).

Regenerated basophilic epithelium failed to grow when transplanted, as did combined epithelium treated with serum ultrafiltrate or hyaluroni-

[3] GREENE, HARRY: Personal communication.

dase. Serum ultrafiltrate was used because Simms demonstrated that this substance found in serum encourages the growth of adult fibroblasts and viruses (*5*). Hyaluronidase was employed because of the possibility of changing the ground substance, either of the transplant or the bed where the transplant was placed.

TABLE 1.—*Autotransplants in adult rats. Combined mucosa*

Treatment	Transplants	Successful transplants	Days
	Number	*Number*	
Controls	4	0	15, 15, 14, 14.
Methylcholanthrene crystals on serosa	3	0	14, 14, 7.
Methylcholanthrene crystals on mucosa	3	0	14, 14, 14.
Methylcholanthrene crystals in area	2	1	13, **13.**
Ligation control	2	0	11, 46.
Ligation plus methylcholanthrene in area	3	2	**11, 46, 46.**
Trypsin 1 percent 10-minute immersion	8	7	**14, 14, 14, 14, 7,** 6, **39, 39.**
Trypsin 1 percent in area	2	2	**15, 15.**
Trypsin 1 percent immersion plus methylcholanthrene in area.	6	3	14, 6, **37, 37, 37, 37.**
Serum ultrafiltrate 10-minute immersion	1	0	6.
Serum ultrafiltrate plus methylcholanthrene in area.	1	0	6.
Hyaluronidase 10-minute immersion	1	0	6.
Hyaluronidase plus methylcholanthrene in area	3	1	6, **37, 37.**
Streptomycin-sulfamylon solution plus serum ultrafiltrate.	2	0	14, 14.
Trypsin immersion plus serum ultrafiltrate immersion.	2	2	**14, 14.**
1,2-benzanthracene crystal in area	4	3	**13, 13,** 37, **37.**
Trypsin plus 1,2-benzanthracene in area	2	2	**15, 22.**
Scarified region	2	2	**19, 19.**
Lysozyme	4	3	**13, 13, 13,** 13.
McIlvaine's buffer	1	1	**14.**
Mucin-dried powder in area	2	2	**15, 15.**

Figures in boldface type indicate days of successful transplants.

After 14 days, successful transplants of mucosal cells obtained by means of auxiliary substances other than methylcholanthrene were composed of either a large cyst or one large cyst with small daughter cysts. The lining cells that stained for mucus were arranged regularly along the walls of the cysts (fig. 7). The cyst fluid stained for mucus had a pH of 7.2 and was slightly viscous. Occasionally small acini of mucous cells were found outside of a cyst. All cells were regular in size and shape.

The largest transplants and those that grew the fastest were obtained with trypsin and either 1, 2-benzanthracene or methylcholanthrene. The former simply produced larger cysts, lined with a luxuriant single layer of mucous cells, while the latter produced cysts and acinous growths containing cells with neoplastic tendencies that became more marked when they were allowed to continue to grow for a period longer than 14 days.

The epithelium of the cyst wall was composed of several layers, and often this thick epithelium grew out into the surrounding connective tissue (Pl. 66, fig. 10, and pl. 67, fig. 11). Large papillary and stalklike growths of these cells were also found extending into cysts (Pl. 68, fig. 12). Many of the cells in the stratified epithelium and in the stalklike growths no longer stained, for mucus and the cells were now irregular in size and shape (Pl. 68, fig. 13). The ultimate fate of these transplants is now being studied.

Why the noncarcinogenic 1,2-benzanthracene caused mucosal cells to grow is of interest, and, moreover, it caused growth of both the pyloric and combined mucosae (tables 1, 2, and 3).

TABLE 2.—*Autotransplantation in adult rats. Pyloric mucosa*

Treatment	Transplants	Successful transplants	Days
	Number	*Number*	
Controls	5	0	14, 14, 14, 13, 13.
Methylcholanthrene crystals in area	2	0	14, 14.
Trypsin 1 percent 10 minutes immersion	1	0	13.
1,2-benzanthracene crystals in area	2	2	**14, 14.**

Figures in boldface type indicate days of successful transplants.

TABLE 3.—*Homotransplants in adult rats. Combined mucosa*

Treatment	Transplants	Successful transplants	Days
	Number	*Number*	
Controls	1	0	6.
Methylcholanthrene on mucosa	2	1	**6**, 69.
Methylcholanthrene on serosa	2	0	6, 69.
Benzanthracene in area	4	0	14, 14, 15, 15.
Methylcholanthrene plus trypsin	4	1	14, 14, 14, **14.**

Figures in boldface type indicate days of successful transplants.

Only two homologous transplantations have been made successfully (tables 2, 3). One was accomplished by placing methylcholanthrene directly on the mucosa and one by means of methylcholanthrene and trypsin. 1,2-Benzanthracene did not produce successful homologous transplantation.

In conclusion, the methods employed demonstrate differences in the capacity of the two types of mucosas of the stomach and the three types of cells to survive injury and to proliferate thereafter. Pyloric mucosa apparently has a greater capacity to withstand injury caused by ischemia in the presence of acid than the combined mucosa. Moreover, the basophilic cell in the combined mucosa is the cell that survives injury and proliferates thereafter, while neither the acid- nor pepsin-secreting cells survives nor proliferates.

Both combined and pyloric mucosa from adult animals require auxiliary

substances for successful transplantation. 1,2-Benzanthracene aids both pyloric and combined mucosa to take, while trypsin and trypsin plus methylcholanthrene are more active auxiliary substances for the growth of combined mucosa. The relationship of anthracenes and mucolytic enzymes to the survival and proliferation of mucous cells is of special interest. The mucous cell is the one that also survives transplantation and continues to grow in the presence of the carcinogen. The mucous cell is the only cell capable of growing into a gastric neoplasm.

The transplants treated with methylcholanthrene are being observed for a long period of time to determine whether they are going to develop into malignant neoplasms.

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(*2*) Kammeraad, Adrian: Development of gastro-intestinal tract of rat; homotransplantation of embryonic and adult gastro-intestinal tract mucosa of rat to anterior chamber of eye. J. Exper. Zool. 91: 45–63 (1942).

(*3*) Smith, William E.: The neoplastic potentialities of mouse embryo tissues; tumors elicited from gastric epithelium. J. Exper. Med. 85: 459–478 (1947).

(*4*) Loeb, Leo: The biological basis of individuality. Charles C. Thomas, Baltimore, Md. (1945).

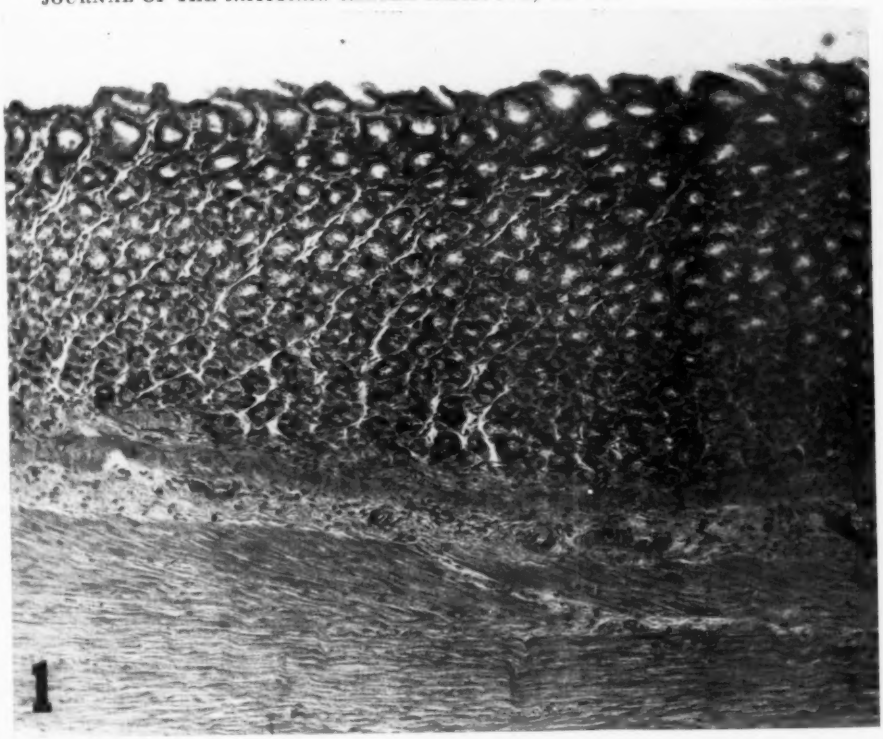
(*5*) Simms, Henry S., and Sanders, Murray: Use of serum ultrafiltrate in tissue cultures for studying deposition of fat and for propagation of viruses. Arch. Path. 33: 619–635 (1942).

Chairman Wangensteen: Thank you, Dr. Howes. Ladies and gentlemen, this concludes the papers, but we will now open the session for discussion of these very interesting papers. Dr. Shimkin, will you start, please?

Plate 62

Figure 1.—Normal combined mucosa.

Figure 2.—Pyloric mucosa after ligation in the blood vessels and in the presence of acid. Normal morphology of cells but intense edema below. Ischemia and the presence of acid has not destroyed pyloric mucosa.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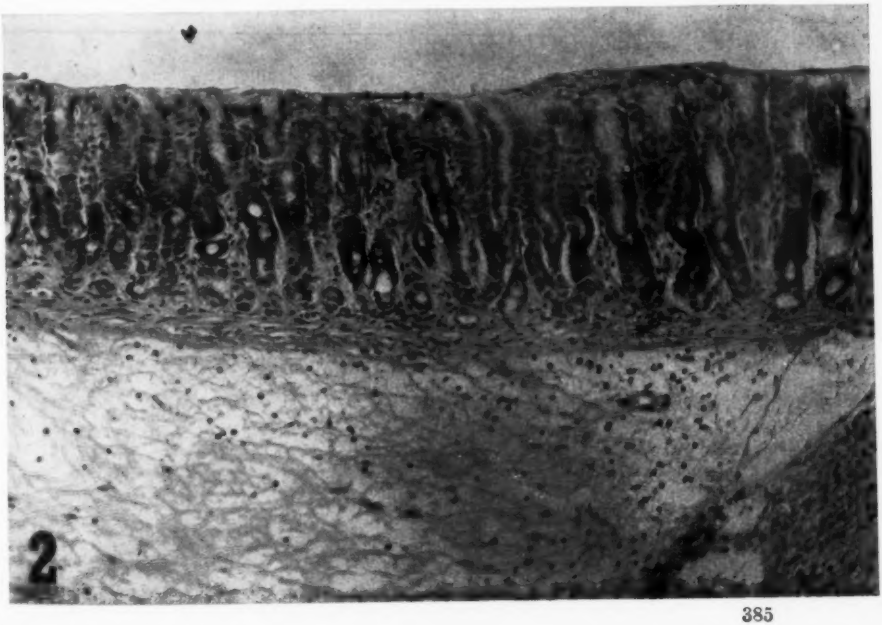
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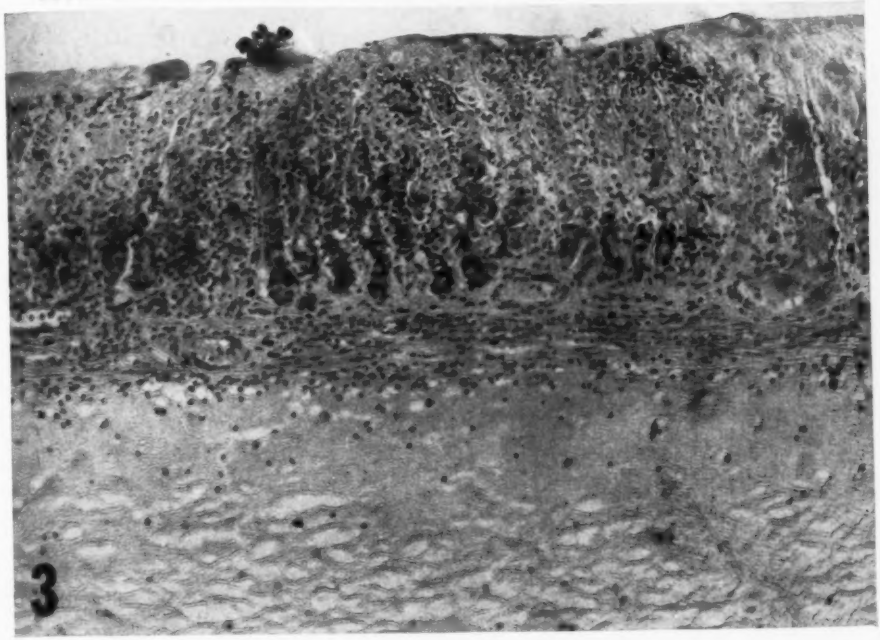
PLATE 63

FIGURE 3.—Partial destruction of combined mucosa with ligation alone 24 hours before.

FIGURE 4.—Complete destruction of combined mucosa with ligation in the presence of acid 24 hours before.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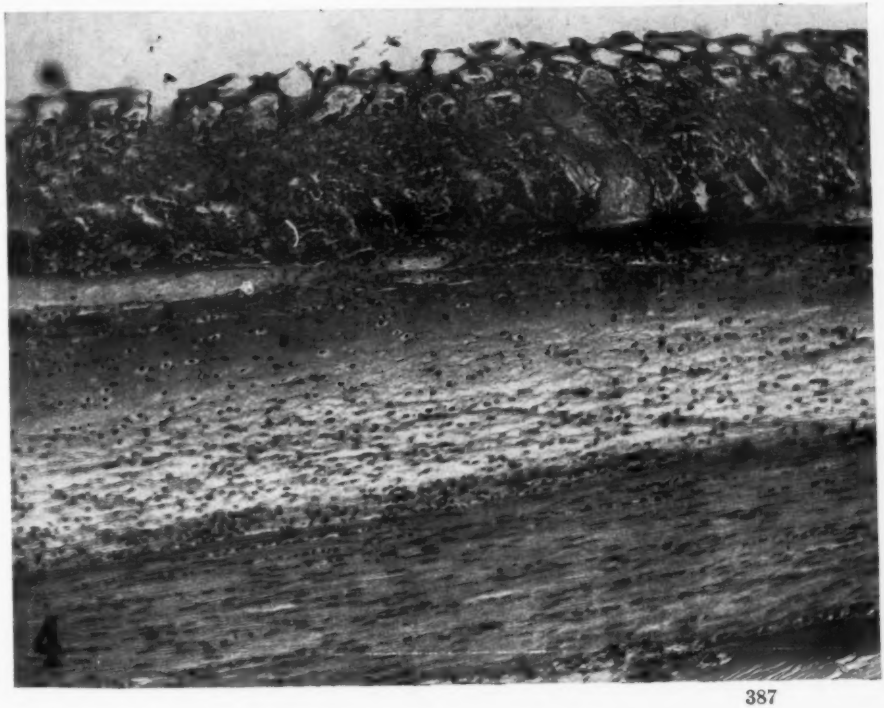
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PLATE 64

FIGURE 5.—Regenerated intestinalized basophilic epithelium following ligation in the presence of acid 3 weeks previously.

FIGURE 6.—Gross appearance autotransplant of combined mucosa treated with 1-percent trypsin and then methylcholanthrene. 39 days. Size 1.5×1×1 cm.

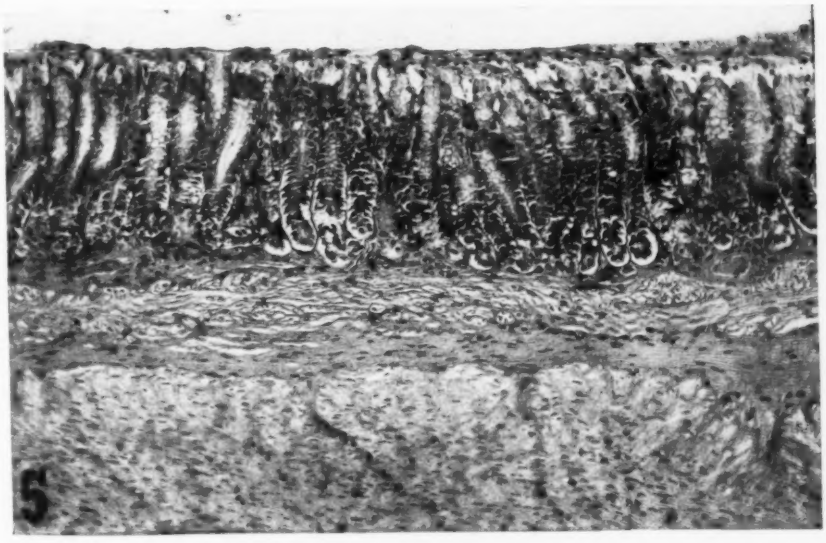
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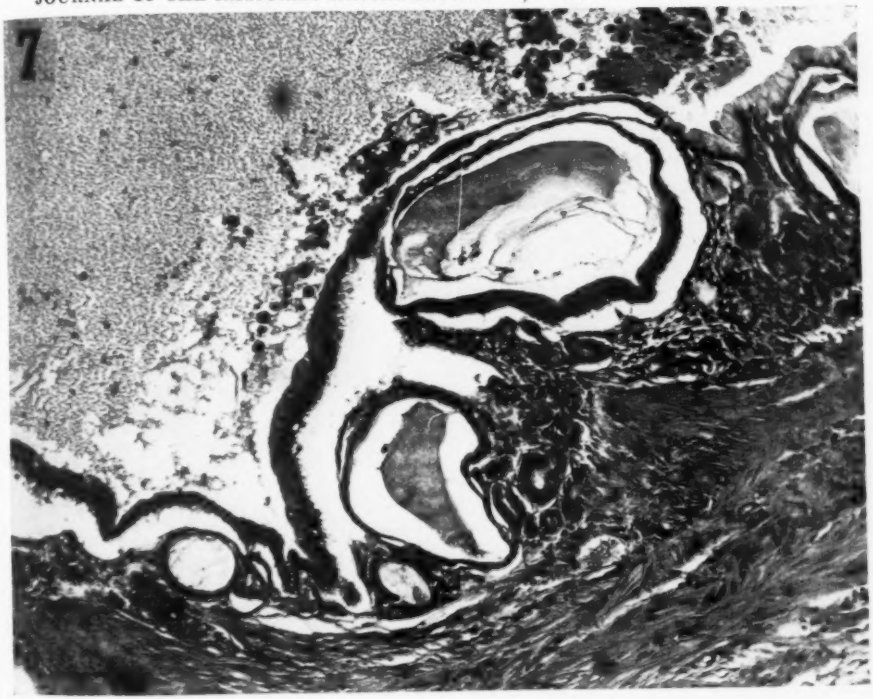
PLATE 65

FIGURE 7.—Autotransplant of combined mucosa. 18 days. Trypsin only.

FIGURE 8.—High power of autotransplant of combined mucosa treated with 1,2-benzanthracene. 13 days. Note mucous droplets.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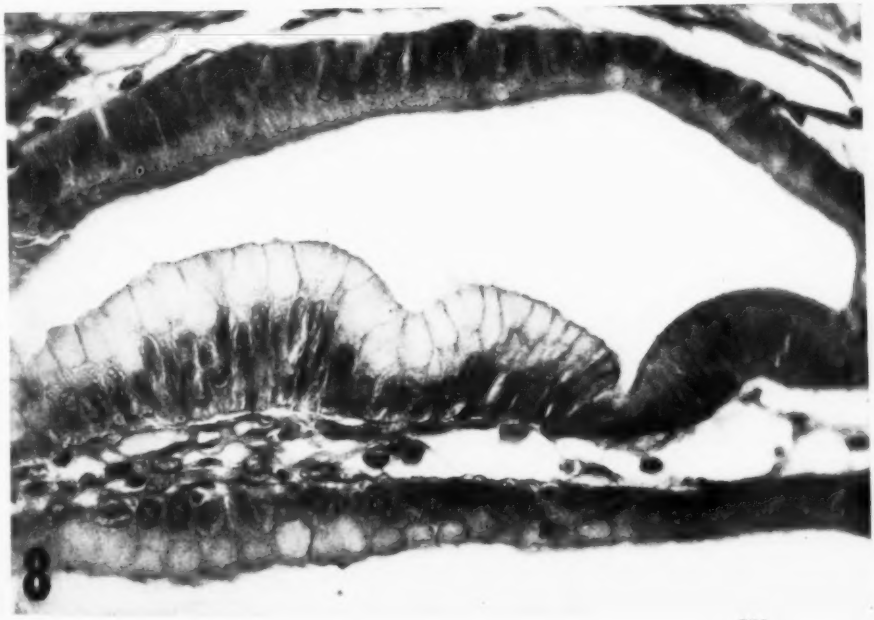
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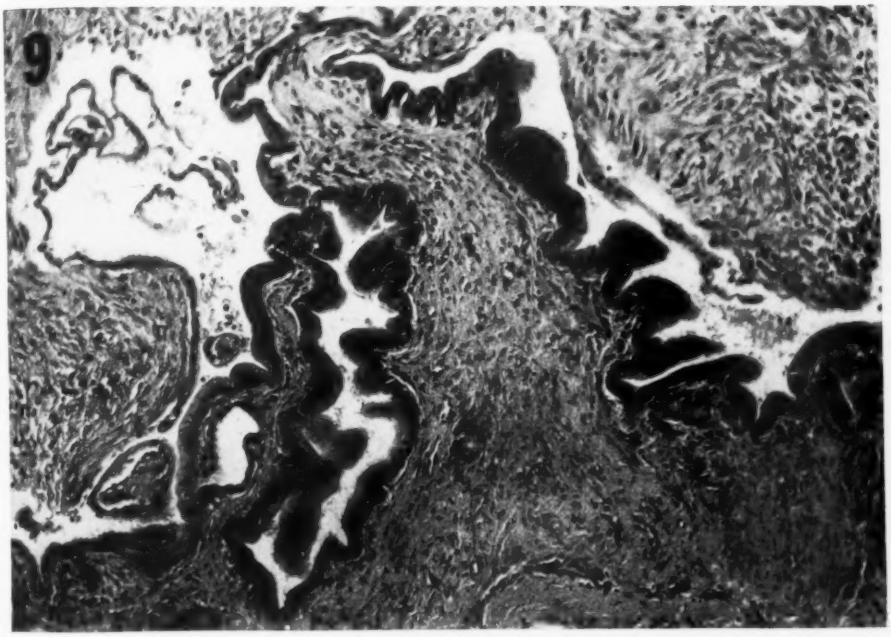
PLATE 66

FIGURE 9.—Autotransplant of regenerated mucosa in the presence of methylcholanthrene. 11 days.

FIGURE 10.—Autotransplant of combined mucosa with 1-percent trypsin plus methylcholanthrene. 37 days. Note thickness of mucosa and disordered arrangement of acini. Growth extending through muscularis mucosa.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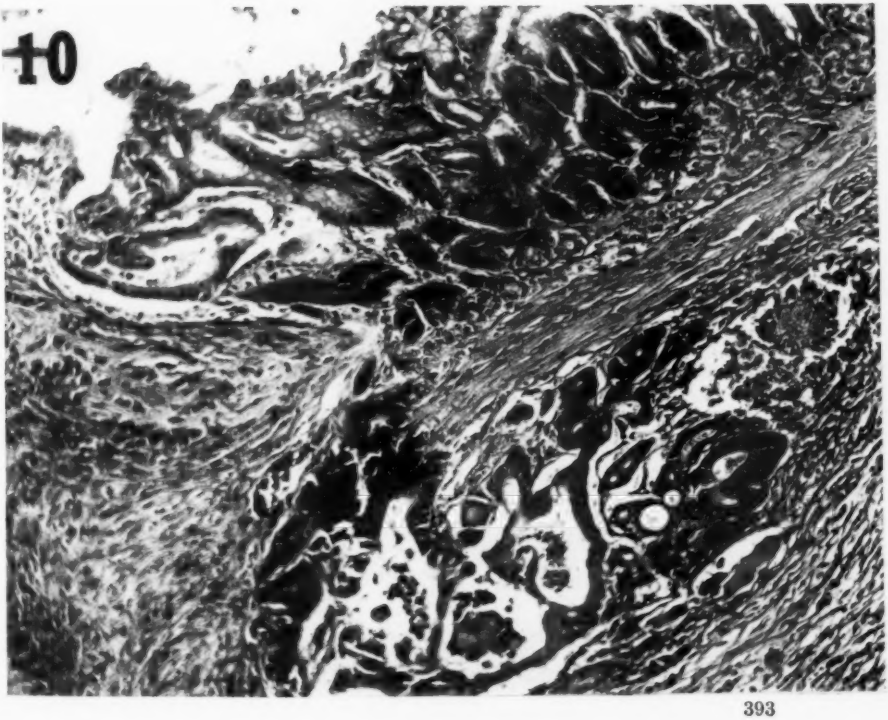
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PLATE 67

FIGURE 11.—Another section of the same transplant as figure 10 showing area of mucosa buried in connective tissue.

11

PLATE 68

FIGURE 12.—Autotransplant of combined mucosa; trypsin, and methylcholanthrene. 49 days. Papillomatous growth extending into a large cystic structure containing glary material resembling mucus.

FIGURE 13.—High-power reproduction of autotransplant. 37 days. Treated with methylcholanthrene and trypsin. Note disordered arrangements of cells and the fact that mucous droplets are no longer seen.

12

13

Dr. Shimkin: About 9 years ago, Dr. Andervont and I did some experiments on the transplantation of gastric mucosa of mice.[1] Saline suspensions of gastric mucosa were injected intravenously into mice of homologous strains, and the animals were killed about 6 months later. We found that about 75 percent of mice injected with suspensions of mucosa from strain I mice having the hyperplastic gastric lesion of this strain showed active growth of the tissue in the lungs. Apparently normal mucosa of other strains showed "takes" in about 5 percent of the animals. We concluded that the gastric hyperplastic lesion of strain I mice had greater capacity for autonomous growth than normal gastric mucosa.

Chairman Wangensteen: Further discussion?

Dr. Shay: I should like to ask Dr. Scharrer what relationship the latent period for the development of the tumor after severance of the nerve has to the balance of the life span or the entire life span of the insect. I realize that it is dangerous to interpret results in one species in terms of another, but one couldn't help but wonder what significance Dr. Scharrer's findings might have for the future of some of our patients whom we are vagotomizing for peptic ulcer, since Dr. Scharrer has shown that the nerve which she cuts in her insects is also an autonomic nerve.

Dr. Scharrer: The insect I used is a long-lived species. The adult life span, that is, counting from the time the adult emerges, may be as long as 18 or 20 months, and the earliest tumor death is perhaps 8 or 9 days. However, I have cases where tumors appear as late as 7 or 8 months after the nerve severance, which would be half the expectancy of the total life-time. Thus, the relationship is somewhat difficult to establish. I also want to say that the various age groups which we tested all succumbed to these tumors. If there is a difference, the older the insect operated on, the more readily these tumors seem to develop, within certain limits.

Chairman Wangensteen: Dr. Scharrer, you said in your presentation that this recurrent nerve was not the analogue of the vagus nerve. I assume you mean the vagus nerve?

Dr. Scharrer: Yes.

Chairman Wangensteen: Is it the analogue of both the sympathetic and the parasympathetic nervous system of the stomach?

Dr. Scharrer: According to the rules, you can never establish any homology between an organ of an invertebrate and of a vertebrate. You could establish analogous organs but, unfortunately, we do not know enough about the question of whether there is a sympathetic and a parasympathetic system in insects. However, there is evidence that two systems very similar to the two in the vertebrates are found in annelid worms, which is a lower group. There is some British work on that subject, and I think only last week some work came out dealing with the physiologic part of the problem, which is interesting, because I suspect that we may find the two components in the insects. But, unfortunately, I could not give you a definite answer to this question. The system is autonomic; that is all I can say.

Chairman Wangensteen: Have you studied by X-ray technique the effect of the emptying time of the stomach on the nerve?

Dr. Scharrer: I have not. It is a good question.

Chairman Wangensteen: Dr. Glick.

Dr. Glick: I would like to make a remark about Dr. Copeland's paper. About 1934, I had occasion to carry out some studies of the esterase enzyme in the gastric mucosa, and it was shown at that time that the epithelial cell region has a considerable concentration of this enzyme. In view of that fact, I wonder if, with tributyrin, lesions aren't due to the hydrolytic products of it.

[1] ANDERVONT, H. B., and SHIMKIN, M. B.: Intrapulmonary transplantation of the adenomatous gastric lesion of strain I mice. J. Nat. Cancer Inst. 2: 151-155 (1941).

In the one experiment shown of growth effects, I believe the ethyl butyrate gave the greatest response and butyric acid the least; that leaves tributyrin in between. I think the control might have been better had it included not only butyric acid but that plus the proper amount of glycerol, because, as I recall, the level at which the compounds were fed constituted an appreciable caloric portion of the diet. The butyrate hydrolyzed faster, and the products absorbed more readily than in the case of the tributyrin, and you might expect the growth increase to be greater with ethyl butyrate.

Chairman Wangensteen: Dr. Strong.

Dr. Strong: I would like to discuss two papers, the first by Dr. Howes who emphasized that the gastric lesion which we have been able to induce in BrS mice by the subcutaneous injection of methylcholanthrene and which has descended to 18 subsequent untreated generations also arises in mucus-secreting neck cells, as affirmed by Dr. Fern Smith. I hope she will discuss this point this afternoon in her paper. I would like to point out that this lesion is also differentiated, and the anomalous lesion has been transplanted subcutaneously, and in the transplant the mucus-secreting neck cell is dominant.

I also point out that another stomach lesion which we have been able to induce in the so-called PBr subline is an anaplastic carcinoma of the forestomach that will also transplant subcutaneously and will grow at a tremendous rate, producing tumors subcutaneously 1 cm. in diameter in 8 days. That tumor is void of mucus. A second type of tumor in mice of the PBr subline induced by a subcutaneous injection of methylcholanthrene is a squamous carcinoma of the forestomach. This tumor is also transplantable.

The other point I would like to make is in relation to Dr. Scharrer's remarks and is this: Anticipating the vagotomy experiments in mice, those were started 2 years ago in New Haven where many mice were vagotomized. It was difficult to keep the animals alive at first after a complete vagotomy, but at the present time the mice can be kept alive. Up to this time vagotomy had no effect, or very little, on the lesion which occurs in the BrS subline. Later, however, further evidence would indicate that vagotomy may have reduced the incidence of the gastric lesion as indicated by gross inspection. These vagotomy experiments, as well as the ones on transplantation, are still being investigated by M. A. Bagshaw.

Chairman Wangensteen: Are there further comments or discussion?

Dr. Code: Again, I am a neophyte in this field, but I would like to ask a question which I hope someone will be able to answer. Since there seems definitely to be a relationship between the ability of the gastric mucosa to secrete acid and the tendency to develop cancer, has a study then been made of the effect of carcinogenic agents on the character of the gastric secretion of animals receiving these agents before they have developed their cancer and while they are developing it?

Chairman Wangensteen: The cooperation or coordination suggested in the vehicle in the carcinogen in the papers presented by Dr. Lorenz and Dr. Stewart indicates that these two papers really merit discussion by someone who works in this field. Dr. Andervont, you aren't beyond paying compliment to your two colleagues, are you?

Dr. Andervont: Dr. Wangensteen, of course those two papers were perfect. I don't believe there is much to discuss. They were presented very clearly.

There were several questions that came to mind in regard to the last three papers which I am sure have resulted from the fact that the speakers didn't have time to present all the data.

I am wondering if Dr. Copeland has considered the lesions in the forestomach of the rat in their relationship to the lesion one sees from a vitamin-A deficiency in this species. I was wondering if, when the animals came to autopsy, you found hair balls in the stomachs of the rats.

Dr. Scharrer's paper was extremely interesting to me from the standpoint of one who is interested in cancer as a problem in biology. It shows that at least she has induced hyperplasia by cutting a nerve. If we agree it is a malignant process, it shows that

we have another cause of malignancy. I think she deserves our congratulations. I should like to point out that she admitted the observation was fortuitous. I believe all of those who work in cancer realize that on rare occasions this does occur.

Dr. Howes' statements regarding transplantation were a little vague to me. Do these transplants grow progressively, and have you taken the transplant from one rat and inoculated another? Can you carry the growths along serially; do they ever regress spontaneously?

Chairman Wangensteen: I see Dr. Shields Warren in the audience.

Dr. Warren: I have very little to add, Dr. Wangensteen. I would like to emphasize two points already mentioned. First, the very great value that comparative pathology offers in the understanding of carcinogenesis. I think we tend to stick too closely to conventional laboratory animals, and it is well worth while to look beyond the rat, mouse, and rabbit to other forms. The other thing I would like to speak of is the question of survival of cells in alien sites within the same mouse. These can occur, and, as has been brought out by Dr. Andervont and Dr. Shimkin, there is also a question whether the mere proliferation in a new site indicates any relationship with the neoplastic process.

Chairman Wangensteen: Dr. Hollander.

Dr. Hollander: May I address a question to Dr. Scharrer? We have been looking for a long time for evidence of some neural innervation of the mucus-secreting cell in the dog and related species. There is definitive histological as well as physiological evidence for the postganglionic innervation of the parietal and peptic cells, but I think the evidence regarding the columnar epithelium is still inadequate. I wonder if you have any evidence regarding the neural innervation of various glandular elements in your animals.

Dr. Scharrer: I have not been successful so far. I have tried to use the protargol method. Unfortunately, the protargol, the essential chemical in it, obtainable now is not the same as it used to be. It came from I. G. Farben and was somehow composed differently. We cannot get the method to work at this time. I have written to somebody in I. G. Farben to get old samples of protargol. That would be one way of getting at that problem. I have tried supravital methods, but I cannot say anything definite with respect to the innervation of the cells.

Dr. Hollander: Dr. Stewart, I would like to ask how much evidence you have regarding the possibility of developing lesions by intramural injections in rats as compared with the mouse.

Chairman Wangensteen: Dr. Hollander, while you are on your feet I would like to ask whether you have any factual data as to why the mucosa in the stomach is so difficult to exhaust of its mucus as contrasted with the colon or the small intestine, reminiscent of the studies made by the Floreys many years back.

Dr. Hollander: I believe that what happens in the course of ordinary digestion, or of very mild topical stimulation in the stomach of a dog and related species, is this: The columnar cells pour out their secretion in a thinly viscous form with a mucin content which is not very high, but the cells themselves remain in situ for the most part. Sometimes this secretion results only from muscular contraction, and the cells are able to respond to this mild type of mechanical stimulation for a very long period of time. During this interval, it is probable that the columnar cells are simultaneously regenerating their stores of secretion in the thecal cavities.

Now, in experiments with mild chemical irritants such as we have been doing for some years past, the stimulating process is a little bit more violent, and we often see columnar cells which have been completely deprived, apparently, of their thecal cavities, as well as some in which the thecal cavities have merely been reduced in size. Of course, these latter cells may be smaller only because they are slowly regenerating secretion after having lost it completely, but I believe this is generally not the case. The method of extrusion of the mucus is one of the problems which we are studying from these same tissue sections which I showed before. It is evidently a different type of process from that observed by Florey in the small intestine and colon.

Chairman Wangensteen: Dr. Stewart.

Dr. Stewart: My associates, Dr. Hare, Dr. Lorenz, Dr. Bennett, and I have an experiment underway in which rats of different strains have received an injection of methylcholanthrene into the wall of the glandular stomach at two sites, one near the pylorus and the other near the limiting ridge.

To date only one carcinoma and four sarcomas have appeared. Eighty percent of the animals autopsied have shown a mass at the site of injection, which we feel is a diverticulum-like lesion. The glandular mucosa shows adenomatous proliferations, but the deeper extensions are limited by a fibrous capsule in which remnants of the musculature of the stomach are frequently incorporated. Despite the atypical hyperplastic appearance of the glandular mucosa, penetration to the serosa does not ordinarily occur. In other words, these growths in most cases are completely surrounded by muscle remnants, fibrous tissue, and peritoneum. All attempts to transplant this lesion to other rats of the same strain have been unsuccessful. We are unwilling to diagnose these lesions as carcinoma because they do not extend onto the peritoneum as does carcinoma in man and in mice.

Chairman Wangensteen: Dr. Ray, of Cincinnati.

Dr. Ray: I just want to throw out an idea which occurred to me in Dr. Howes' paper. We classify chemical compounds as carcinogens or noncarcinogens. Perhaps we ought to have another subcarcinogen or semicarcinogen there. His interesting paper shows a way of possibly studying and determining this subclassification.

Chairman Wangensteen: Any other discussion? Dr. Shay.

Dr. Shay: With reference to Dr. Stewart's paper, it may be of interest to add at this time information concerning our own efforts in the past 2 years to produce adenocarcinoma in the rat's stomach. At the conclusion of the Gastric Cancer Conference 2 years ago, the consensus was that the mucus layer covering the gastric mucosa may serve as an important barrier against the production of gastric glandular cancer in the experimental animal. We attempted to break that mucus layer by producing ulceration of the antrum of the rat's stomach by feeding young rats a calcium-free diet. Such a lesion, we also reasoned, in the process of attempted healing would have young cells available which could be exposed to methylcholanthrene administered by stomach tube. The carcinogen dissolved in olive oil was instilled daily for 6 days of the week in Wistar strain rats grown in our colony (2 mgm. per rat).

We failed to produce any gastric lesions after continuing the above procedure for more than a year. We did, however, obtain a high incidence of adenocarcinoma of the breast in female rats. These cancers are prevented by castration done at the time methylcholanthrene administration is started. Of special interest is the fact that the spontaneous incidence of adenocarcinoma of the breast in the Wistar rat is practically nil in the age period in which we obtained an incidence in over 50 percent of the females. Dr. Stewart has very kindly reviewed the slides of the tumors obtained in our animals.

Chairman Wangensteen: Dr. Copeland.

Dr. Copeland: Dr. Glick, I would like to answer your question. I think that the statement that these lesions may be due to some hydrolytic product of tributyrin rather than the material itself is possible but probably does not afford the best explanation for its action. Ethyl butyrate and other esters of butyric acid have been fed to a large number of animals at varying levels, and there have been various levels of butyric acid and tributyrin. In each case, however, the tributyrin seems to have the most harmful effect. The effect of ethyl butyrate on the rat's stomach is mild compared with that of tributyrin.

In response to Dr. Andervont's question, I would like to state that the lesions that we have observed in rats fed tributyrin are much more severe than those produced in our laboratory on Papenheimer type diets or on the experiments with vitamin A. The lesions are more extensive and involve more of the layers of the stomach wall. So far as hair balls are concerned, we have encountered them but we do not believe that they have any important relation to causation of the lesions.

Dr. Stewart has examined the slides of the tissues that I presented to you here sometime back.

Chairman Wangensteen: Dr. Howes, I would like to ask whether you feel there is any likelihood that the extension of tissue transplantation you have spoken of may be of any use in other biologic transplantations employed by surgeons in extending the range of such techniques.

Dr. Howes: I would like to answer Dr. Shimkin's question first and call attention to the fact that autotransplantation of adult cells did not occur unless auxiliary substances were used. We have not done the experiment with mice. Of course, it should be. I am sorry to have missed your paper about the grinding up of the stomach. As you know, other tissues have been transplanted with carcinogens. These experiments could have been referred to, but I thought best to mention only the work done on the stomach. I would like to point out again the lack of success with homologous transplantations, even with auxiliary substances. The strains of rats used were both Wistar and Sherman rats. We have found no difference varying with strain so far.

To answer Dr. Andervont: From the histological point of view, whenever the substance employed was not a carcinogen, the growth of mucous cells appeared benign. We have not subtransplanted the carcinogenic growth, except in two instances. One grew, but we may have carried over the carcinogen. Repeated subtransplantation will have to be done when we get more growths.

In answer to Dr. Code's question concerning regenerated and the pyloric mucosa: As you know, many believe atrophic gastritis precedes the development of the gastric neoplasm. Clinically the neoplasm arises most frequently toward the pyloric end of the stomach where it might originate either in pyloric or intestinalized mucosa. The basophilic mucosa that regenerates after the combined effect of ischemia and acid resembles that seen in atrophic gastritis. Regenerated mucosa alone did not transplant, but with the carcinogen it transplanted more frequently in the few experiments conducted than the combined mucosa. On the other hand, pyloric mucosa thus far was more influenced by benzanthracene than methylcholanthrene. Many more experiments will still have to be done to prove the real differences between these two types of mucosae. From the point of view of the origin of the neoplasms of the stomach, however, it is interesting that the work thus far suggests they respond to different stimuli. We have no other laboratory method that I know of, to chemically distinguish pyloric from regenerated mucosa. We have killed rats in the laboratory with vagotomies done 1 year before, and the mucous membranes looked normal. The experiments to date point out two aspects of the problem of carcinoma of the stomach, namely, the capacity of the mucous cell to live and proliferate with an altered blood supply and, second, originally emphasized by Dr. Stewart's work, namely, that the carcinogen must remain in contact with these cells long enough to distort their proliferative activity. To answer Dr. Wangensteen, the use of auxillary substances as judged from these experiments should help the surgeon to do transplantations, and I want to thank him for pointing out this possibility.

Chairman Wangensteen: If there is nothing further to be added, we will be dismissed, gentlemen, to convene here again at 2 o'clock.

Morning session adjourned at 12:50 o'clock.

MONDAY AFTERNOON SESSION
December 13, 1948

Spontaneous lesion of stomach in strain I mice---------- HOWARD B. ANDERVONT

Lesions of the gastric mucosa in Strong strain NHO mice------- HENRY S. KAPLAN

Studies on gastric neoplasia in mice. The histogenesis and influence of some endocrine factors---------- FERN W. SMITH and LEONELL C. STRONG

Etiologic implications of the racial incidences of gastric cancer---- PAUL E. STEINER

The Washington State Tumor Registry and its role in problems regarding gastric cancer and cancer research---------- H. DAVIS CHIPPS

An attempt to identify likely precursor groups of gastric cancer----------
DAVID STATE, DAVID GAVISER, THOMAS D. HUBBARD, Jr., and OWEN WANGENSTEEN.

Photofluorography in the detection of gastric cancer----------
JOHN F. ROACH, ROBERT D. SLOAN, and RUSSELL H. MORGAN

Cytologic study of gastric aspirate for carcinoma cells----------
HENRY C. BRYANT, WILLIAM R. CRAIG, and H. MARVIN POLLARD

The cytohistological study of gastric washings---------- HOWARD L. RICHARDSON

Use of the esophagoscope for obtaining biopsies of gastric tumors----------
CECIL O. PATTERSON

Acid-phosphatase activity in the gastric content of patients with carcinoma of the stomach---------- GEORGE W. CHANGUS and CHARLES E. DUNLAP

Further studies on metabolic changes in patients with gastric cancer----------
F. HOMBURGER, NELSON F. YOUNG, AURELIA POTOR, JACK B. TRUNNELL, BENEDICT J. DUFFY, Jr., MARY L. PETERMANN, and C. P. RHOADS.

The Conference reconvened at 2:00 o'clock p. m., Monday, December 13, 1948, Dr. Alexander Brunschwig, presiding.

Chairman Brunschwig: Ladies and gentlemen, the meeting will come to order. The first three papers will be taken as a group with discussion at the end of the third paper. The paper by Dr. Steiner will not be given; he is not here. The next seven papers will be taken as a group with discussion after Dr. Dunlap's paper and then discussion after Dr. Homburger's paper. The first paper this afternoon is Spontaneous Lesion of Stomach in Strain I Mice by Dr. H. B. Andervont of Bethesda, Md.

SPONTANEOUS LESION OF STOMACH IN STRAIN I MICE [1]

By HOWARD B. ANDERVONT, *National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.*

The I strain of mice was originated by Dr. L. C. Strong (*1*) who, between 1925 and 1927, started 12 strains of mice from "unpedigreed pairs of mice . . . primarily from the colony of Dr. W. E. Castle of Bussey Institution, Harvard University". Only 4 of these strains, namely I, F, L, and N are now in existence. Another strain (strain JK) which was derived from strains J and K of the 12 original strains is also in existence (*2*). Strong (*3*) hybridized strains JK, N, and CBA to procure the NHO strain of mice which "have acquired a new characteristic, cancer of the stomach, following the subcutaneous injection of methylcholanthrene".

Strong selected five recessive characters, namely: pink-eyed, dilute, brown, nonagouti, and piebald to establish the I strain. The subline of the strain maintained at the National Cancer Institute also carries chinchilla. These are descendants of a litter procured from the Roscoe B. Jackson Memorial Laboratory. The stomach lesion was first encountered in strain I mice during 1936 in one of the first animals to be autopsied in this laboratory and was subsequently found in virtually all mice of the strain except those dying early in life (*4*).

The lesion has been adequately described elsewhere (*5*). It arises in the pyloric chamber and is discernible macroscopically because the hypertrophied mucous membrane produces a thickening of the stomach wall. It begins at the junction of the forestomach and pyloric chamber, partially encircles the esophageal opening, and extends along both curvatures. It is normally widespread and symmetrical in distribution, but sometimes the gastric lumen is filled with flat nodular masses. Microscopically the lesion is an adenomatous overgrowth of the glandular elements of the mucosa together with development of atypical epithelium with some penetration into the gastric wall. The lesion is not considered to be malignant because it develops symmetrically, conforms to a general pattern in all mice, and is not generally transplantable. Thus far, metastases have not been seen.

It is easily found in virtually all strain I mice that live 8 months or longer and is on the average, more pronounced in the males (*6*, *7*). It is not communicable to other mice and is apparently not caused by an infectious agent. Dietary studies show that the lesion is not caused by a deficiency of vitamin A but is more pronounced in mice fed a diet containing roughage than in those maintained on a relatively bland diet. Since it is not seen in F_1 hybrids derived by reciprocal matings of strain

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

I and other strains, the tendency to develop the lesion seems to be recessive. Efforts to transplant the lesion subcutaneously or intraperitoneally into strain I mice have been unsuccessful, but intravenous injection of saline suspensions of minced hyperplastic gastric mucosa results in growth of the lesion in the lungs of strain I mice and in F_1 hybrids derived from strain I (*8*).

For the purpose of this conference, it is important to point out that lesions in the pyloric portion of the mouse's stomach are not limited to strain I. Less extensive lesions are found in older mice of strains C3H, dba, and C57 black as well as commercial mice (*4*, *5*). Strong (*3*) speaking of strain N mice stated "which also develops adenomatous hyperplasias, although very rarely. In the strain N mice the adenomatous hyperplasia is more localized in origin than is the broad, widespreading type that develops in the I mice spontaneously".

Finally, Bullock and Curtis (*9*) found hypertrophic gastritis in rats that had ingested large numbers of eggs from the cat tapeworm, *Taenia taeniaeformis* (*crossicollis*). Blumberg and Gardner (*10*) also studied the stomachs of rats following a heavy infestation by *Cysticercus fasciolaris* of 235 to 272 days' duration. The pronounced lesion was confined to the glandular portion of the stomach and appeared to be adenomatous. Thus, lesions of the pyloric chamber of the stomach are known to occur in a rodent other than the mouse.

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Chairman Brunschwig: The second presentation will be by Dr. Henry S. Kaplan, San Francisco, on Lesions of the Gastric Mucosa in Strong Strain NHO Mice.

LESIONS OF THE GASTRIC MUCOSA IN STRONG STRAIN NHO MICE [1] [2]

By HENRY S. KAPLAN, M. D., *Department of Radiology, Stanford University School of Medicine, San Francisco*

INTRODUCTION

Experimental research on gastric cancer has long been delayed by the rarity of this lesion in animals (*1*). Stewart and Lorenz (*2*) were the first investigators to succeed in the induction of unequivocal adenocarcinoma of the mouse stomach by intramural injection of carcinogenic hydrocarbons. More recently, Strong and coworkers (*3*, *4*) reported the induction by remote injection of methylcholanthrene and also the spontaneous occurrence of cancers and other lesions of the pyloric stomach in mice of his outcrossed NHO strain. Opportunities for evaluation of the pathology of these lesions have been limited, and the only descriptions to date are those of Strong (*3*) and McPeak and Warren (*5*). The present report is offered primarily as an extension of their observations.

EXPERIMENTAL

Three groups of mice of strain NHO (subline br,s) were studied.[3] One group was exposed at 1–2 months of age to whole-body roentgen irradiation for a total dose of 1000 r in 12 daily treatments;[4] another received a single subcutaneous injection of 0.1 cc. of 1.0 percent methylcholanthrene in benzene at 2 months of age; and the third group was maintained untreated. The animals were identically caged and had free access to Purina Laboratory Chow pellets and water. They were maintained until death or until obviously moribund. Unless excessive autolysis had occurred, complete autopsies were performed, including careful macroscopic examination of the stomach.

The stomach was routinely opened along the greater curvature, rinsed clean, and pinned flat on cardboard strips prior to fixation in Zenker's-formol. Serial sections of large portions of the stomach were obtained in many instances when gross lesions were detected and occasionally when they were not. Single sections of areas of the remaining stomachs were made except where autolysis was far advanced. The other viscera also were routinely subjected to gross and histologic examination. In a few instances, when localized gastric lesions were observed in freshly killed

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[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] These studies were initiated in the Department of Radiology, Yale University School of Medicine, with the aid of a grant from the Jane Coffin Childs' Memorial Fund for Medical Research; they were completed at the National Cancer Institute, Bethesda, Maryland.

[3] Breeders from which mice for this experiment have been obtained were very generously made available from his colony by Dr. Leonell C. Strong of the Department of Anatomy, Yale University School of Medicine.

[4] Physical factors were: 130 KV., 5 ma., 30 cm. distance, 3 mm. Al added filtration, output 42 r per minute.

mice, small fragments were excised from them and inoculated by trocar subcutaneously into other mice of the same strain.

OBSERVATIONS AND RESULTS

Except for the fact that large cysts bulging into the pancreas were not observed, the gastric lesions encountered corresponded very closely with those described by McPeak and Warren. Focal abnormalities in the gastric mucosa were frequently found either as isolated lesions in an otherwise normal area or in close relation to large polyplike elevations. They include small cysts near the base of the mucosal layer (Pl. 69, fig. 1), small islands of apparent squamous metaplasia (Pl. 69, fig. 2), large, irregularly branching glands (Pl. 70, fig. 3), and focal areas in which nuclear polarity in the glandular epithelium is reversed. The hyperplastic, proliferative lesions usually take the form of large, flat, sessile polyps protruding into the lumen of the stomach, generally just above the pylorus on the lesser curvature side (Pl. 70, fig. 4). On histologic study, these reveal an actual increase in thickness of the mucosal layer, as distinguished from simple mucosal folds (Pl. 71, fig. 5).

The glands are markedly irregular and distorted and tend to give rise to cystic expansions near the base of the mucosa, some of which protrude through a gap in the muscularis mucosae and grow out into the submucosa, elevating the mucous layer and giving the lesions their flat polypoid configuration (Pl. 71, fig. 6). Epithelial irregularities such as variation in height and staining properties and the presence of intraluminal papillary projections are common in the submucosal cysts (Pl. 72, figs. 7, 8). Rarely, the cysts extend through an apparent gap in the true muscularis into the subserosa, but invasion of contiguous organs or the peritoneal cavity was not observed in this study. By examination of serial sections, it has invariably been possible to trace the cysts up through the muscularis mucosae to the glands of the mucosal layer (Pl. 73, fig. 9). Occasionally, nests of cells which seem invasive are encountered in the submucosa (Pl. 73, fig. 10), but again on serial sections there are seen to be irregular outpouchings from submucosal cysts that have been cut through at their extreme periphery.

Metastasis from a gastric lesion has not been observed in this study. After subcutaneous transplantation to other NHO mice, the polyplike lesions may give rise to a localized swelling which reaches a diameter of almost 1 cm. in about 1 week, followed by prompt, complete, and apparently permanent regression. The localized swelling, on gross examination of cross-sections, seems to be due to an accumulation of secretions and to a peripheral inflammatory reaction, and the mucosa in the transplant seems to undergo rapid degeneration.

A variety of tumors and nonneoplastic lesions occurred in other organs. Polyps of the squamous forestomach were seen in only two instances. Lymphoid tumors similar to those previously described in other strains (*6*) were an important cause of early death in the irradiated group. Local fibrosarcomas, sometimes accompanied by epidermoid or anaplastic carcinomas, were common in the carcinogen-injected groups before 300 days of age. The incidence of gastric lesions in these two groups cannot be evaluated accurately because allowance must be made for animals dying prematurely from these causes and others exhibiting excessive autolysis. Lung tumors were encountered in all three groups but were most regularly seen, and usually multiple, in the methylcholanthrene-treated group. This group also had a higher incidence of a peculiar neoplastic or granulomatous lesion, designated purely for convenience as reticuloendotheliosis, characterized by infiltration of the mesenteric lymph nodes, liver, and spleen, and less often of the lungs, kidneys, stomach, and intestinal wall, and the other lymph nodes, by masses of large mononuclear cells with an admixture of leukocytes, lymphocytes, and occasional giant cells.

Another lesion common to all groups was a massive enlargement with reddish or purple discoloration of the mesenteric and occasionally of other lymph nodes, apparently due to the development of enormous blood-containing vascular spaces which tended to crowd out normal lymphoid elements. Female mice of all groups were prone to develop ovarian cysts of either serous or hemorrhagic character and extremely large size. Two mice (one irradiated, one control) developed paralysis or staggering gait in the hind legs, and dissection of the vertebral column revealed in each case a solitary tumor arising from the meninges. These are still under study by Dr. Harold L. Stewart. Sporadic hepatomas occurred in all groups.

The incidence of these various lesions in relation to survival time in each group is presented in table 1. After rough corrections are made in the two treated groups for the number of animals dying early from lymphomas, fibrosarcomas, and other induced tumors, the incidence of gastric lesions appears to be about equal to that in the controls. However, in the methylcholanthrene-injected group, there may have been some acceleration of development of the gastric changes. Since the latter were rarely a cause of death, however, they may have been present undetected for a relatively long time in the older animals. Determination of the actual latent period must await further studies on animals killed at serial intervals.

TABLE 1.—*Incidence of various lesions in relation to survival time*

Group	Age at death	Mice	Stomach				Lymphoma	Lung tumor	Fibrosarcoma	Reticuloendothelioma	Hemolymph nodes
			Polypoid lesion	Atypical mucosa	Negative	Unknown					
	Days	*Number*									
X-ray	300	8	0	0	4	4	8	0	0	0	1
	300–500	3	1	1	1	0	1	0	0	0	0
	500	15	8	1	0	6	0	10	0	8	5
Total		26	9	2	5	10	9	10	0	8	6
Control	300	0									
	300–500	3	1	0	0	2	0	0	0	1	1
	500	10	8	0	0	2	0	3	0	1	8
Total		13	9	0	0	4	0	3	0	2	9
M C	300	10	1	5	1	3	1	3	9	2	1
	300–500	16	6	5	2	3	0	13	4	8	10
	500	2	2	0	0	0	0	2	0	1	1
Total		28	9	10	3	6	1	18	13	11	12

DISCUSSION

This strain of mice is remarkable for the multiplicity of neoplasms and related conditions to which it is susceptible either spontaneously or after exposure to chemical carcinogens or irradiation. Of these, only the gastric lesions and the meningeal tumors are apparently unique for this strain.

The proliferative gastric lesions undoubtedly resemble tumors in certain respects, but as observed in this experiment they would appear to lack certain attributes of neoplastic behavior such as true invasiveness, metastasis, and transplantability to homologous hosts. However, final evaluation of their significance must await detailed studies by qualified pathologists. Such studies are justified by the fact that no other tumor or tumorlike process occurs spontaneously with practical frequency in the glandular gastric mucosa of other strains or species, with the possible exception of the hyperplastic lesions long known to occur in strain I mice (*7*).

There is little doubt that the gastric lesions of strain NHO mice are histologically quite different from the several varieties of human gastric adenocarcinoma. Recently, an isolated instance of congenital cystic hyperplasia of the stomach in an adult human, clinically simulating carcinoma, has been reported (*8*). The hyperplastic lesions illustrated in that report bear a distinct resemblance to those described here.

SUMMARY

A variety of mucosal abnormalities, including polypoid proliferative lesions and cysts, were observed in the glandular stomach of mice of Strong's strain NHO. The incidence of these spontaneous lesions did not appear to be influenced by roentgen irradiation or the remote injection of methylcholanthrene. True invasion and metastasis were not observed, and subcutaneous transplantation of a few of the polypoid lesions failed to yield progressively growing tumors in homologous mice. Several other types of neoplasms were also noted.

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Chairman Brunschwig: The third paper will be by Dr. Fern W. Smith of Columbia, Mo., Studies on Gastric Neoplasia in Mice.

PLATE 69

FIGURE 1.—Isolated cyst in otherwise apparently normal glandular mucosa of strain NHO mouse. ×150.

FIGURE 2.—Focal squamous metaplasia extending down from surface of prepyloric region into branching cryptlike structures. ×350.

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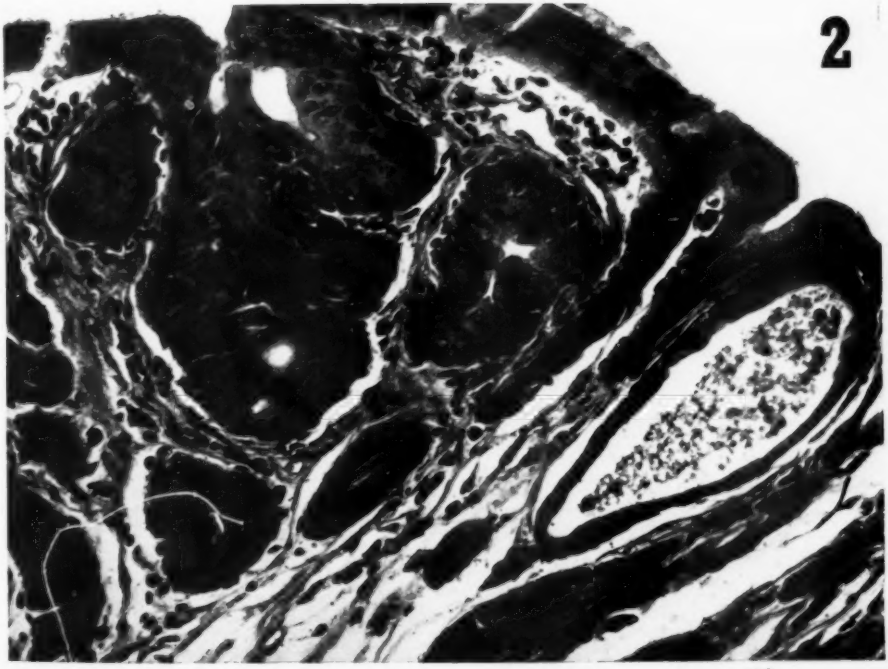
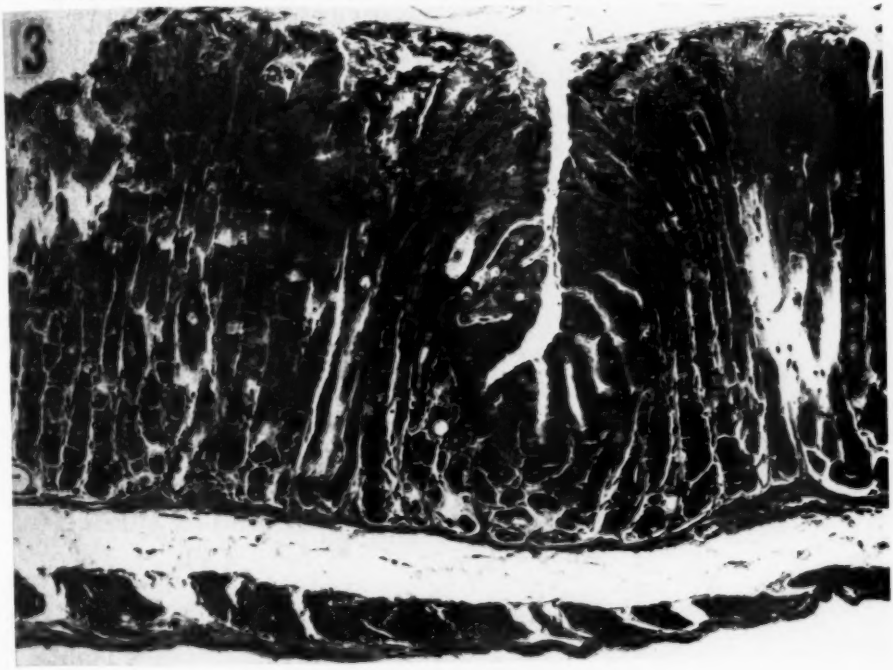


PLATE 70

FIGURE 3.—Arborizing glandular structure in a zone of tall crypts. ×150.

FIGURE 4.—Large polypoid lesion protruding into the gastric lumen near the pylorus. It contains numerous irregularly branching glandular structures which are not arranged in the orderly manner of normal crypts. ×50.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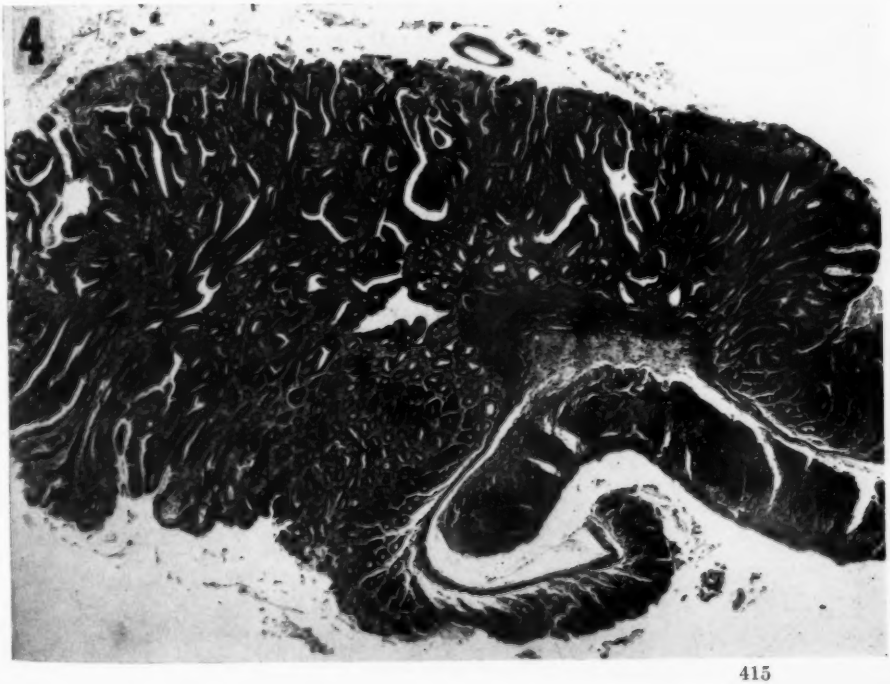
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PLATE 71

FIGURE 5.—Normal rugal fold sectioned transversely for comparison with figure 4, showing a central stalk of submucosal connective tissue and regular glandular architecture. ×50.

FIGURE 6.—Elevation of mucosal layer by proliferating, cystic submucosal extensions to form a sessile pseudopolypoid intraluminal mass. ×20.

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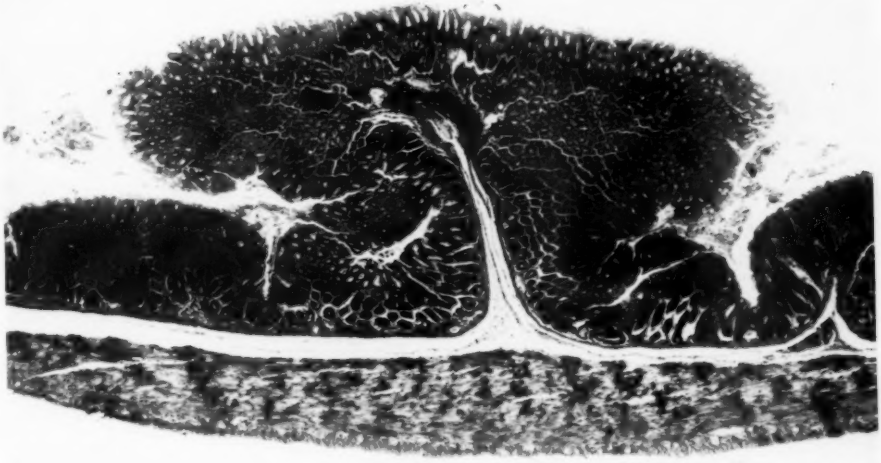

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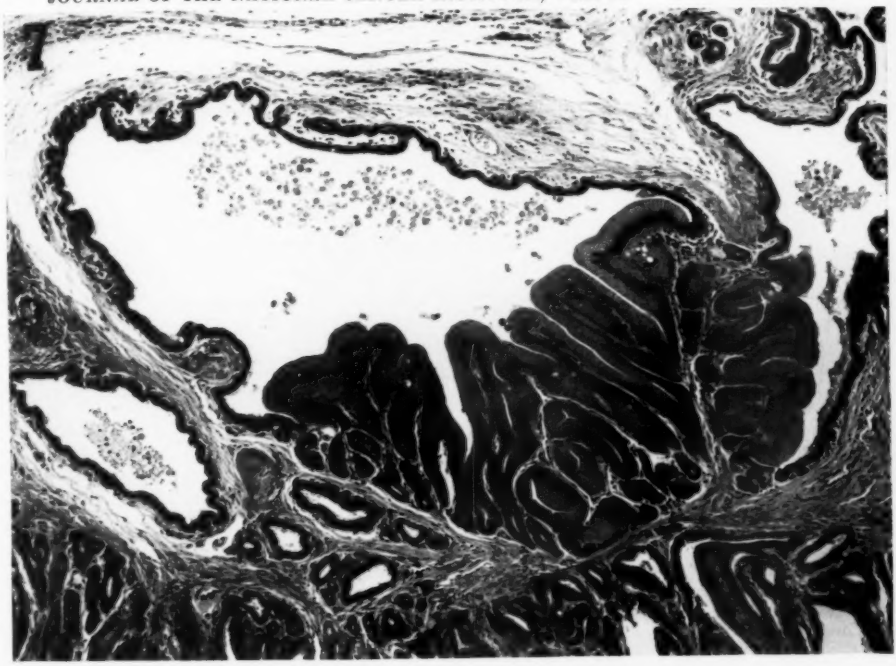
PLATE 72

FIGURE 7.—Higher magnification of submucosal cysts to show variations in height and staining reactions of epithelium and irregular papillary ingrowths. ×100.

FIGURE 8.—Cystic structure extending from zone of atypical mucosa down through the muscularis mucosae into submucosa, surrounded by chronic inflammatory reaction. ×100.



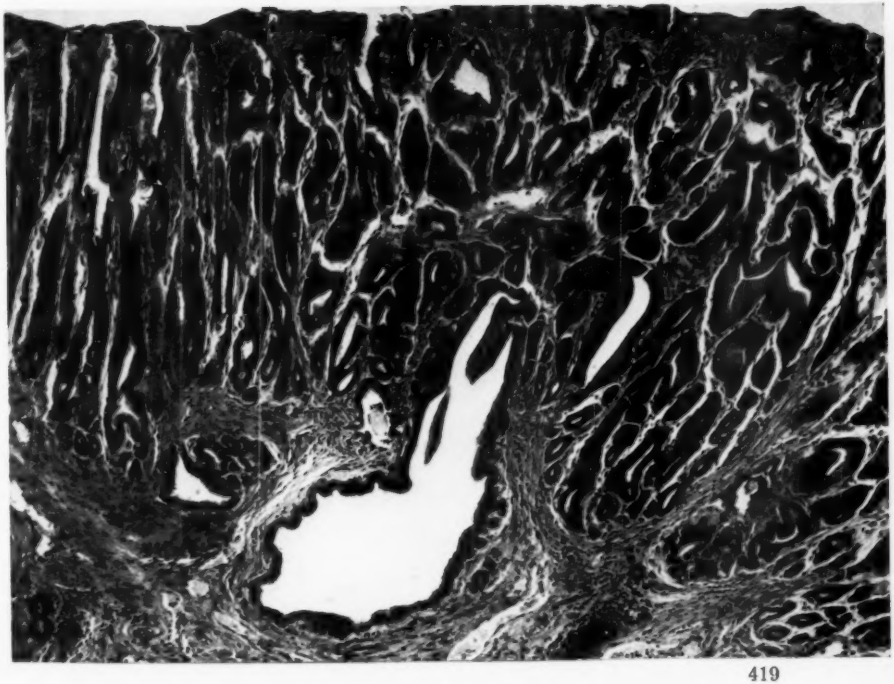
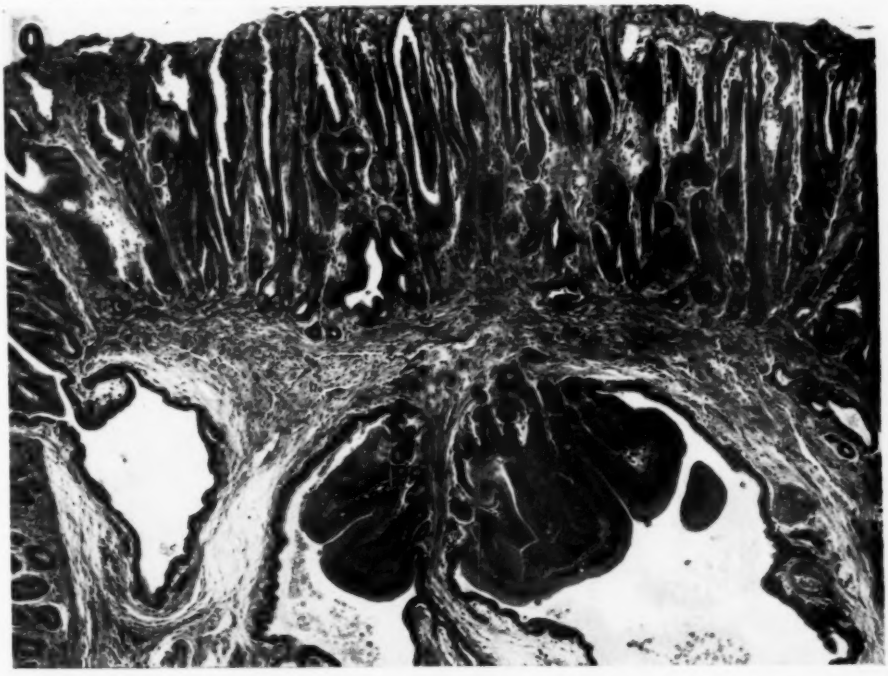
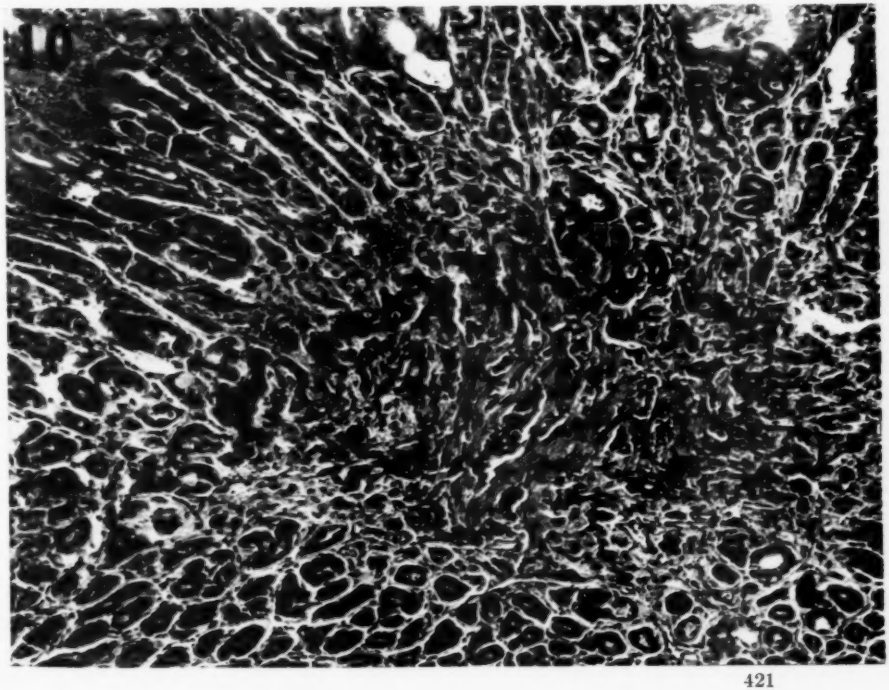


PLATE 73

FIGURE 9.—Cystic structures from another mouse, similar to those in figures 7 and 8. The branching cyst on the left can be traced up through the mucosa to the surface. ×85.

FIGURE 10.—Nests of epithelial cells enmeshed in submucosal connective tissue, simulating a focus of true invasion. Study of serial sections demonstrated that these cells were directly continuous with outpouchings from small cystlike downgrowths. ×150.





STUDIES ON GASTRIC NEOPLASIA IN MICE. THE HISTOGENESIS AND INFLUENCE OF SOME ENDOCRINE FACTORS [1] [2]

By FERN W. SMITH [3] and LEONELL C. STRONG, *Department of Anatomy, Yale University, New Haven, Conn.*

The production and development of spontaneous gastric neoplasia in mice of the BrS stock suggested the investigation of the role played by endocrine secretions. At approximately four hundred days of age, the gastric lesion was present in 100 percent of the male animals and in but 60–70 percent of the female mice.[4] Because statistics of the incidence of gastric carcinoma in the human population also showed a sex difference in favor of the male,[5] the effects of castration upon the growth of the lesion in mice was the initial approach in the present study.

The mice of the BrS stock are descendants of animals which had received 1 mg. of methylcholanthrene dissolved in 0.1 cc. sesame oil at 60 days of age. Four groups of animals were used in the present experiments: males, intact and castrated; and females, intact and castrated. The total group of castrated mice include three groups: those which had been gonadectomized at 1–2 days, at 3–4 weeks, and at 2 months of age. The mice were maintained on Purina Laboratory Chow in an air-conditioned room. Representative animals in each of the groups, 4 months and older, were selected at random for study of the developmental aspects of gastric and other lesions. Tissues were fixed either in Bouin's or in Zenker's fluid and were stained routinely with haematoxylin and triosin. The gastric tissues were also stained for mucin by several methods (mucicarmine, mucihaematin, potassium ferrocyanide).

The normal mouse stomach is divided into two portions: a nonglandular forestomach lined with squamous epithelium and a glandular portion containing the secretory elements. These two areas are separated from one another by a limiting ridge composed of squamous epithelium. The entire glandular portion of the stomach is lined with tall columnar epithelial cells that secrete mucin. The glands in the pyloric portion are also mucin-secreting cells as determined by mucin staining techniques, whereas the parietal cells and chief cells are distributed throughout the body of the stomach.

The histopathology of the lesion has been studied extensively in early

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[1] Presented at the Fourth Conference on Gastric Cancer sponsored by the National Advisory Cancer Council of the National Cancer Institute in San Francisco, Calif., December 13–14, 1948.

[2] This study was aided by a grant from the Donner Foundation.

[3] Present address: Department of Anatomy, University of Missouri, Columbia, Mo.

[4] STRONG, L. C.: Unpublished data.

[5] MCDONALD, E.: Cancer Record Registry, Connecticut State Department of Health. Personal communication.

stages by McPeak and Warren [6] and others.[7] The lesions in the gastric mucosa of the mice of this series have been characterized by abnormal proliferation of the gastric epithelium including adenomas, adenocarcinomas, and squamous-cell carcinomas. There are three loci of tissue proliferation: (1) the forestomach in which the lesion and its extensions to other parts of the body consisted of squamous cells, (2) the limiting ridge where the tumor with or without its metastases were composed of undifferentiated cells, and (3) the pyloric portion of the stomach. The following material is limited to those lesions found in the latter position.

Small nodules, readily palpable by rolling the gastric walls between the index fingers and thumb, and larger nodules visible by direct examination were both detectable in the pyloric region of the stomach. The vascularity of the region was usually increased, and occasionally there were adhesions between the nodules and the pancreatic tissue. Small, firm, yellow nodules characteristic of fat necrosis were observed in some of the older animals in the gastrosplenic and gastropancreatic ligaments.

Histological sections of normal pyloric tissue showed the mucosa arranged to form short gastric pits lined by cells in the cytoplasm of which mucin was demonstrable.

Deviations from the normal histological picture appeared frequently. Localized accumulations of leucocytes, chiefly neutrophils, plasma cells, and lymphocytes occurred at the pylorus. Small leucocytic infiltrations, most frequently on the lesser curvature, became conspicuous in the connective tissue below the muscularis mucosa. More extensive lesions involved the tunica propria which later became edematous. Simultaneously with the mucosal reaction, local hyperplasia of glandular tissue occurred and extended to encircle the lumen at the pylorus. The hyperplastic mucosa showed an increased tortuosity of glands and the presence of small mucin filled cysts. The low columnar type of epithelium was modified into that composed of tall columnar epithelial cells. Long folds of mucosa frequently extended into the gastric lumen. Growth was often sufficient for these folds to assume the appearance of small papillomas. Small lesions rarely penetrated through the muscularis mucosa.

Associated with some of the larger glandular proliferations of mucosa was a slight eversion of the muscularis mucosa. Accumulations of leucocytes occurred in the adjacent submucosa. Serial sections of such lesions indicated the penetration of the glandular tissue through and beyond the muscularis mucosa. The connection with the gastric lumen was maintained. These diverticulae were cystic and were lined by epithelial cells varying from low cuboid to tall columnar. Mucin was present in the lumena of the smaller cysts and in the cytoplasm of the lining cells. In some cases the glandular formations extended into and through the muscle layers to the serosal membrane, but there was no invasion of adjacent organs.

[6] McPeak, E., and Warren, S.: The pathology of gastric carcinoma in mice. J. Nat. Cancer Inst. 7: 309-311 (1947).

[7] Strong, L. C., Collins, V. J., and Durand, E. H.: A genetic analysis of induction of tumors by methylcholanthrene. IV. The probable remote induction of various types of gastric lesions. Cancer Research 3: 21-28 (1943).

Three animals of the total number observed showed an adenomatous hyperplasia of the gastric mucosa, a condition defined as a proliferation of the epithelium but without extension beyond the muscularis mucosa. These animals were not included in the tabulated data which consisted only of those lesions extending beyond the muscularis mucosa (table 1).

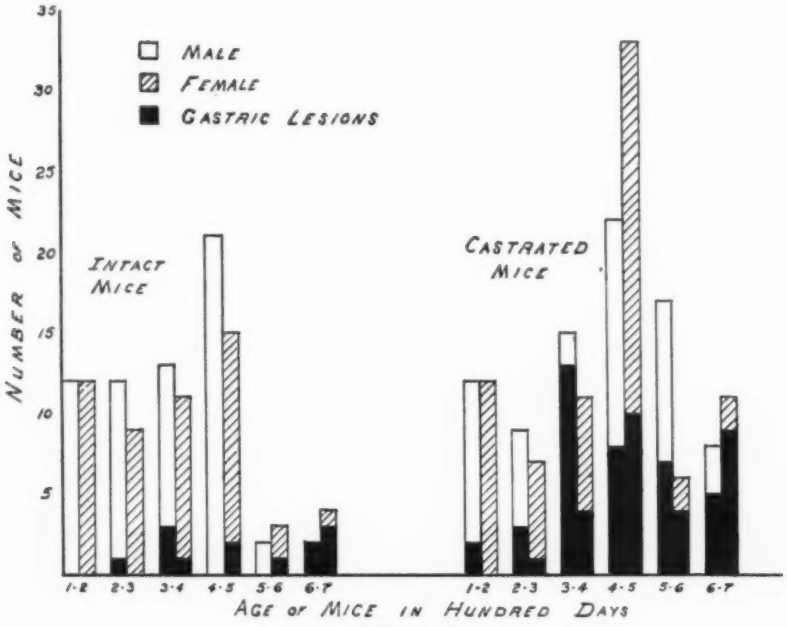
TABLE 1.—*Incidence of spontaneous gastric lesions of the pyloric stomach in control and castrated mice of the BrS stock*

Mice	Female			Male		
	Mice	Lesion	Lesions	Mice	Lesion	Lesions
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Control	54	7	12. 9	62	6	9. 6
Castrated:						
1–2 days	8	2	25. 0	3	1	33. 3
3–4 weeks	44	17	38. 6	46	18	39. 1
2 months	28	9	32. 1	34	19	55. 9
Total	80	28	35. 0	83	38	45. 7

Gastritis was observed in both male and female mice and more frequently in the animals of the castrated than of the intact group. The incidence of the gastric lesion in the control intact mice was low in both the male and female groups (table 1). In castrated mice of both sexes the incidence of invasive lesions was higher than in the intact animals. The incidence was also higher in the castrated males than in the castrated females (text-fig. 1).

The number of animals in certain age groups was small (table 2). The mortality of the colony was highest in the 400–500-day age group, decreasing effectively the relative number of animals in the older age groups. In the intact mice, the lesions in the female were observed in older age groups than in the male. The difference in incidence was slight. Among the castrated animals, the lesions in the male were observed as early as 5 months, again earlier than in the female mice and in greater numbers than in the female mice. The peak of the incidence among the males occurred at 300–400 days, in spite of the greater mortality in the 400–500-day age group. Among the castrated female mice, the incidence of lesions increases with age.

The animals in neither the intact nor the castrated group showed pathology of the adrenal glands which could be associated with the gastric lesion. Mice in the older age groups showed senile changes characteristic of adrenal cortical tissue of mice in which subcapsular cells proliferate. Among the castrated mice, there was some evidence of decrease in width of the cortex, fatty depletion, and brown degeneration in the cortico-medullary zone. These changes were not consistent and were not correlated with the presence of the gastric lesions or the sex of the animal. Four medullary tumors were observed among the castrated mice; 2 in mice with the gastric lesion; 2 in mice without the lesion; 3 in female mice; 1 in a male.



TEXT-FIGURE 1.

TABLE 2.—*Tumor incidence in intact and castrated BrS stock mice in the pyloric stomach showing extension below the muscularis mucosa*

Days	Intact males			Intact females			Castrated males			Castrated females		
	Tumors	Mice	Percent	Tumors	Mice	Percent	Tumors	Mice	Percent	Tumors	Mice	Percent
		Number			*Number*			*Number*			*Number*	
100–200		12			12		2	12	6. 2		12	
200–300	1	12	8. 2		9		3	9	33. 3	1	7	14. 2
300–400	3	13	23. 0	1	11	9. 0	13	15	83. 3	4	11	36. 3
400–500		21		2	15	13. 3	8	22	36. 6	10	33	30. 3
500–600		2		1	3	33. 0	7	17	41. 1	4	6	66. 6
600	2	2	100. 0	3	4	75. 0	5	8	62. 5	9	11	81. 8
Total	6	62	9. 6	7	54	12. 9	38	83	45. 7	28	80	35. 0

The kidney and submaxillary glands were also examined for evidence of the presence of endogenous androgen; both organs increase in weight in androgen-treated mice as well as show histological alteration. Neither tissue yielded information to indicate abnormal quantities of androgen which might be correlated with the presence of the gastric lesion.

In summarizing, the following was apparent: Gastric lesions at the pylorus of the stomach in this subline of the BrS stock may be characterized by (1) gastritis involving a leucocytic infiltration beneath the

muscularis mucosa and subsequently throughout the tunica propria, (2) an adenomatous hyperplasia of the gastric mucosa occurring locally or encircling the lumen at the pylorus, (3) hyperplasia of the mucosa and the submucosal extension of invasive glandular tissue. The proliferated epithelium was composed chiefly of mucin-filled cells. Because lesions of similar size were found in animals of all age groups and no metastases were observed, it is assumed that the lesion grows slowly and infiltrates only locally.

Gastric lesions occurred with greatest frequency among castrated male animals. The incidence was also high among the castrated female mice. The data indicated that the absence of the sex hormones have influenced the development of the gastric lesion in mice.

Chairman Brunschwig: Does anyone want to start the discussion on the three papers on neoplasia of the mouse's stomach? Dr. Strong?

Dr. Strong: I do not want to complicate this gastric carcinoma situation any more but to point out that this one lesion just anterior to the pylorus is the only lesion in the BrS and its many sublines that has become hereditarily established by a mutation on the brown chromosome. The other two types of tumors have not as yet been hereditarily established, but I do feel that if large enough numbers of mice could be established without the further injection of methylcholanthrene, that also could be done.

I would like to point out the influence of genetics on gastric lesions since this is not represented on this symposium. There is genetics involved in this pyloric lesion. We have produced evidence that the gastric lesion is linked with coat color by the mutation on the brown chromosome. We have obtained a mutation from brown to black hair pigmentation 8 times in the series of mice which have been injected subcutaneously with methylcholanthrene.

In all cases, the B1 subline, characterized by a mutation from b to B, which was obtained by mutation of the BrS subline, the late appearance in the gastric lesion with its many histological variations has been delayed more than 200 days.

In other words, the brown segregates retain the susceptibility of the brown ancestry, whereas the black mutants have acquired a new characteristic which has been acquired as a recessive, and we have evidence that the process of crossing over has occurred between the brown locus and the locus that determines the gastric lesion under induced conditions.

This means, further, that when a mutation occurs following the subcutaneous injection of the methylcholanthrene, at least three loci on the same chromosome have mutated at approximately the same time. Two of those mutations have been from a recessive to the dominant condition, and one of them has been a recessive from the dominant condition. This means that methylcholanthrene has not induced germinal changes by chromozomal breakage, but there must be some other biochemical explanation of why methylcholanthrene or one of its metabolites is able to induce germinal changes in the chromosomes of mice.

Chairman Brunschwig: Does anyone else wish to discuss these three papers?

Dr. Howes: May I ask Dr. Smith for some interpretation of what she feels the results are in her particular experiments as to what castration did? Was it local change, or what is her feeling about the change?

Dr. Smith: I think it is rather hard to evaluate the lesion as we have found it. We did not find histological changes in the adrenal and submaxillary glands or kidney, in which we hoped to get evidence of some differences in the level of androgen and estrogen secreted in these animals. We know that we have induced in these animals

a modification of the endocrine balance by removing the usual source of androgens and estrogens, and we feel that we must investigate more carefully the role of the gonadotropic and adrenotropic hormones, in themselves, and the manner in which they may influence this lesion. We are now progressing with that study.

Editor's note.—The following paper, Etiologic Implications of the Racial Incidences of Gastric Cancer, by Paul E. Steiner was to have been presented at this point in the program. Although urgent business prevented Dr. Steiner from reading the paper, it is being included in the proceedings of the conference.

ETIOLOGIC IMPLICATIONS OF THE RACIAL INCIDENCES OF GASTRIC CANCER [1] [2]

By PAUL E. STEINER, M. D., *professor of pathology, University of Chicago, Chicago*

It has long been recognized that the incidence of gastric cancer differs in the various races of mankind, although the exact size of the difference remains uncertain. The cause for such racial differences is also not clear. Are they due to differences in constitution, perhaps hereditary, or in the environment? Much current research on gastric cancer is based on the assumption that extrinsic environmental factors are important in causation. Any data, whether positive or negative, that contribute to the problem should be of interest.

It is the purpose of the present paper to present new data (1) on the relative incidence of gastric cancer among persons of different races, living under many similar environmental conditions, and (2) on the question of whether observed differences between races are explainable on a hereditary or environmental basis by comparing these racial groups as immigrants with their corresponding racial sedantes. Does the incidence of gastric cancer in a race change after its members migrate from one environment to another? If it does not, then either gastric cancer is constitutionally determined, or the new environment is equal to the old as far as stimuli for gastric cancer are concerned. On the other hand, if it changes, then at least that proportion of this tumor incidence which has changed is explainable on an environmental basis. Although there may, after migration, be some change in characteristics generally regarded as controlled by heredity (*1*), such changes are gradual and not of high order.

SOURCE OF THE MATERIAL

The present data were obtained from the necropsy records of the Department of Pathology of the Los Angeles County Hospital. This material is exceptionally well suited for such a study and in some respects is unique. Here it is possible to compare, side by side, the incidence of gastric cancer in members of the three great races of mankind, namely, Caucasoid, Negroid, and Mongoloid, in a large population. All cases (35,293) were accurately diagnosed at necropsy by modern and uniform criteria in the years 1918 to 1947. Furthermore, the patients, regardless of race, were from the same economic level, namely, the indigent ill of

[1] This paper was to have been presented at the Fourth Conference on Gastric Cancer held at Stanford University, December 13, 1948. Although urgent business prevented Dr. Steiner from attending the conference and reading the manuscript, it is included in the proceedings of the conference.

[2] The author is deeply grateful to the Research Committee of the Los Angeles County Hospital Staff for permission to make this study and for the splendid cooperation and encouragement of the Committee and of the Pathology staff, especially Dr. E. M. Butt.

Los Angeles County. Finally, the same standards of skill in treatment and in diagnosis during life and after death apply equally to the members of all races. These people differ from each other in race and in some environmental factors. There is little difference in the percentage of necropsy permits. There may be a racial difference in tendency to enter the hospital, if the disease is known by the patient or his relatives to be fatal and terminal.

In the analysis of the material, the Caucasoids and Negroids have been retained as racial groups without further subdivision. It was found that the Mongoloid peoples could be divided to advantage into three subgroups: Japanese, Chinese, and Filipinos. This left unclassified the second largest group in the population, the Mexicans. In this category are included Spanish-Americans, Mexican immigrants and their descendants, and descendants of the original inhabitants of southern California who adopted a Spanish culture many years ago. The exact proportions in the group of persons of each of these derivations is unknown, but the second is probably the predominant source of the group. Obviously, racially this is a heterogeneous group containing both Caucasoid and Mongoloid elements, the latter probably greatly predominating. For present purposes, however, these peoples are combined into and analyzed as one group. Regardless of the justification of this decision from the ethnologic viewpoint, the group shows some special features with regard to incidence of tumors, and especially of some types of neoplasm.[3]

ANALYSIS OF ORIGINAL DATA

Among all necropsies (35,293) there were 741 cases of gastric cancer.[4] It was the commonest malignant tumor. The 26,515 necropsies on Caucasoids disclosed 604 cases of carcinoma of the stomach. This was 2.3 percent of necropsies and 11.8 percent of all malignant tumors in this race. Numerically this was the commonest form of cancer. Some of the other races showed interesting and significant differences when compared with the Caucasoids. Differences were found in incidence, in numerical rank, and in sex ratios. The statistical data are summarized in tables 1 and 2.

The Chinese had no examples of this disease and the Filipinos only one case, but these figures are probably not significant as these necropsy populations were small. By contrast, however, 21 cases which were found in Japanese, whose population was only slightly larger, were all the more surprising and significant.

Carcinoma of the stomach was the commonest cancer in Mexicans, accounting for 1.3 percent of all deaths and 13.7 percent of all deaths due to tumor. There was no significant difference from Caucasoids in incidence in the age 21 to 90 years, whether tested for the sexes combined or for each separately. In Mexicans, gastric cancer accounted for a smaller

[3] These data will be presented in detail in a forthcoming monograph on racial cancer.

[4] Throughout this paper cancer is used as synonymous with malignant neoplasm. It includes carcinomas, sarcomas, leukemias and related lymphatic diseases, intracranial tumors, and miscellaneous malignant tumors

percent of all deaths than in Caucasoids (1.3 percent compared with 2.3 percent), but this is explained by the higher mortality in the younger age groups in the former from tuberculosis and other causes.

TABLE 1.—*Comparative racial incidence of carcinoma of the stomach by decades, Los Angeles County Hospital (1918–47)*

Age	Caucasoid			Mexican			Negroid			Japanese		
	Autopsies	Gastric cancers	Gastric cancer	Autopsies	Gastric cancers	Gastric cancer	Autopsies	Gastric cancers	Gastric cancer	Autopsies	Gastric cancers	Gastric cancer
Years	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
21–30	1, 446	8	0. 5	671	3	0. 4	200	1	0. 5	22	1	4. 5
31–40	2, 230	22	1. 0	711	13	1. 8	360	3	1. 2	40	3	7. 5
41–50	3, 353	61	1. 8	748	13	1. 7	397	5	1. 3	48	1	2. 0
51–60	4, 801	161	3. 4	651	21	3. 2	383	9	2. 3	57	10	17. 5
61–70	5, 428	180	3. 3	441	17	3. 8	312	11	3. 5	22	5	22. 7
71–80	4, 272	142	3. 3	243	13	5. 3	112	5	4. 5	5	1	20.
81–90	1, 228	28	2. 3	68	1	1. 5	36	0	0.	0	0	0.
91–100	55	1	1. 8	3	0	0.	4	0	0.	0	0	0.
Over 100	5	0	0.	1	0	0.	1	0	0.	0	0	0.
Total	22, 818	603	2. 6	3, 537	81	2. 3	1, 805	34	1. 9	194	21	10. 8

TABLE 2.—*Comparative racial incidence of carcinoma of the stomach at the Los Angeles County Hospital (1918–47)*

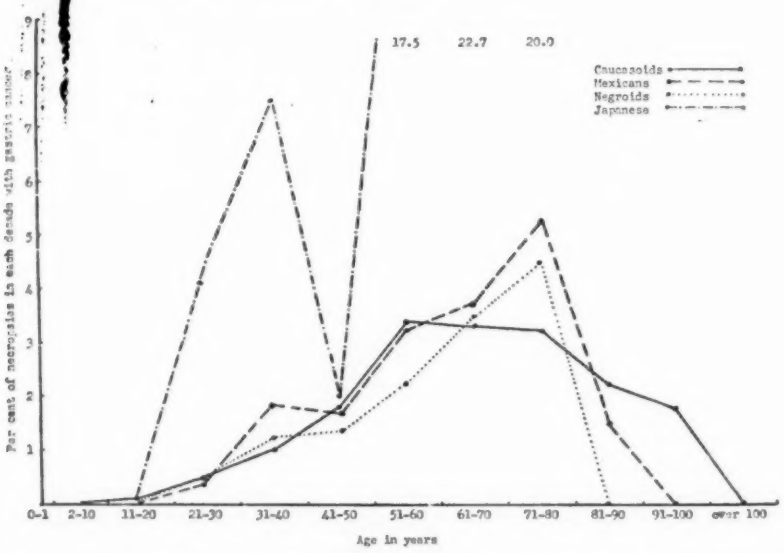
Race	Total autopsies	Autopsies showing a malignant tumor	Carcinoma of the stomach	
			Gastric cancers of all cancers	Corrected ratio of occurrence in males to females
	Number	*Percent*	*Percent*	
Caucasoids	26, 515	19. 3	11. 8	1:0. 5
Mexicans	6, 150	9. 6	13. 7	1:0. 5
Negroids	2, 236	12. 7	12. 0	1:1. 3
Japanese	239	24. 3	36. 2	1:0. 1
Chinese	70	12. 8	0	----------
Filipino	83	13. 2	9. 8	----------
Totals	35, 293	17. 2	12. 2	----------

When Negroids were compared with Caucasoids, some distinct differences in incidence were found. In Negroids, gastric cancer fell to second place, being surpassed by uterine cancer. Negroids had a significantly lower ($P=0.05$) incidence of gastric cancer in the age group 21–90. This was due to a significantly lower incidence in Negro men ($P=0.02$) and not in women ($P=0.5$). This cancer was commoner in the Negroid female than in the male, an observation which, if general, merits further attention.

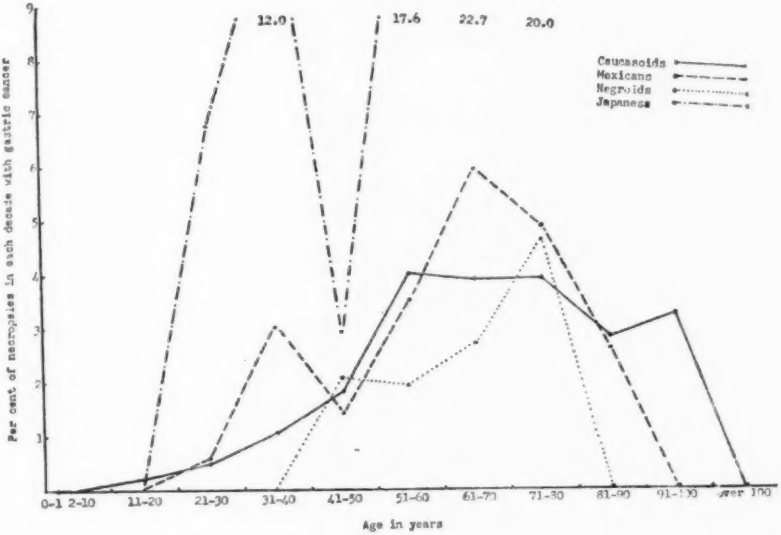
The corrected ratio of incidence in males to females was 1:1.28. This sex ratio is in contrast with the corresponding figures of 1:0.145 in Japanese, 1:0.50 in Mexicans, and 1:0.51 in Caucasoids.

The Japanese had significantly more gastric cancer than the Caucasoids in the age 21–90 whether the comparison is made for both sexes ($P=0.001$) or for the men alone ($P=0.001$). There were not enough Japanese women to warrant separate analysis. In the Japanese, gastric cancer was the commonest tumor, comprising 36.2 percent of all fatal neoplasms. This was over three times the figure for Caucasoids.

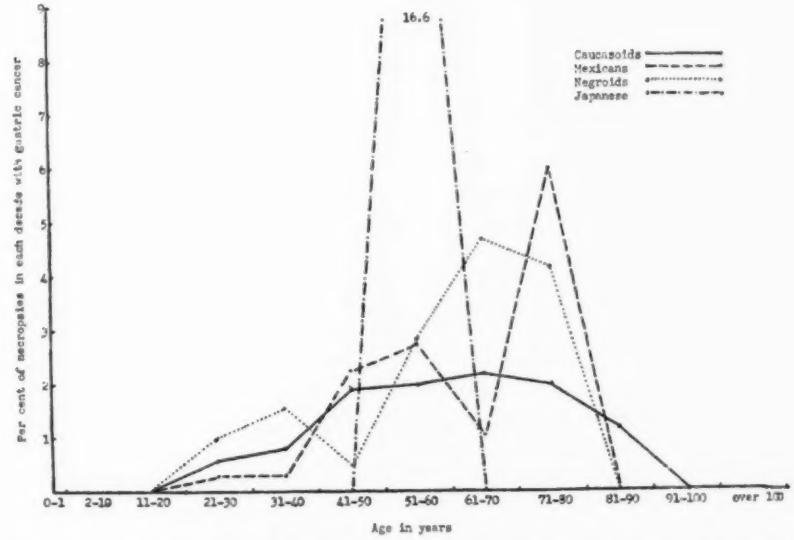
In this necropsy series, the ratio of males to females was different for each race. The average age at death was also different. Mortality in infancy and childhood was much greater in the Mexicans than in the Caucasoids, while the other racial groups were intermediate between these with the exception that in early adult life the mortality rate in Filipinos exceeded even that in the Mexicans. For these reasons, the data were analyzed in terms of the percentage of persons of each race who were autopsied in each decade who had gastric cancer (table 1 and text-fig. 1). This procedure corrects for the inequality in death rates at the different ages. Finally, the same data were analyzed separately for each sex (text-figs. 2 and 3) to correct for the great differences in sex ratios in the various races. This was necessary because gastric cancer is a form of cancer in which the sex ratio is unequal. The results are as already indicated. In table 2 no corrections have been made for racial differences in average age at death or for ratio of males to females. For these reasons, these data must be interpreted with great caution.



TEXT-FIGURE 1.—Incidence of carcinoma of the stomach at necropsy by decades in four races.



TEXT-FIGURE 2.—Incidence of carcinoma of the stomach at necropsy by decades in four races. Males.



TEXT-FIGURE 3.—Incidence of carcinoma of the stomach at necropsy by decades in four races. Females.

COMPARISON OF IMMIGRANTS WITH SEDANTES

Caucasoids

The data on incidence of gastric cancer presented here from the Los Angeles County Hospital agree in general with those based on necropsies from other general hospitals in the United States and in some parts of Europe. Carcinoma of the stomach usually ranks first; it comprises about 12 percent of all fatal neoplasms (although the figure is higher if it is expressed in terms of carcinomas only), and it makes up about 2 percent of all necropsies. A comparison of these Caucasoids in Los Angeles with their sedantes is not possible because the details on time of immigration and geographical origin in Europe are not available. It is becoming increasingly more certain that geographical differences in the incidence of this form of tumor exist in Europe. Comparisons in descendants of these various European peoples in the present material are, however, not possible either with each other or with their sedantes. It is, therefore, impossible to state whether the incidence of gastric cancer in Caucasoids has changed subsequent to migration to America with its attendant changes in environment.

Mexicans

No data on incidence of gastric cancer based on a large number of modern necropsies have come to our attention from Mexico. The older report by Hoffman (*2*) compiled from clinical diagnoses in a number of Mexican cities invariably shows gastric cancer to rank second and after cancer of the female genital organs. It is impossible to make a comparison, using his data, with the present Los Angeles data to see if the incidence has changed in the new environment.

Data are badly needed from Mexico on necropsies performed by the pathology department of a large university general hospital (but otherwise unselected) according to current modern standards.

Negroids

The reported data on tumor incidence on natives in Africa show wide differences. The incidence varies with region in Africa, source of the data (whether clinical, surgical, or necropsy diagnoses), age of the population, and other factors. However, several facts almost invariably stand out. These are (1) the high incidence of primary liver cancer and (2) the low incidence of primary gastric cancer.

Since the differential diagnosis between primary liver cancer and primary gastric cancer with metastases to the liver may be clinically difficult, the present analysis deals only with reports of necropsies.

Strachan (*3*) reported upon 3,851 necropsies from the General Hospital, Johannesburg, of which 1,622 were on Europeans, 328 on Eurafricans, and 1,901 on Bantus. He found the incidence of gastric cancer in Europeans to be 2.6 percent of all necropsies, a figure which resembles that here presented from Los Angeles (2.3 percent) in whites. In Bantus, however, the incidence was only 0.31 percent, in contrast to 1.5 percent

in Negroids in Los Angeles. This low figure from Africa for gastric cancer in Bantus is not indicative of low incidence of other types of cancer. For example, in Strachan's necropsies the incidence of primary liver cancer was 0.25 percent in Europeans and 1.9 percent in Bantus. Corresponding figures from Los Angeles are 0.24 percent for Caucasoids and 0.18 percent for Negroids. Data from other parts of Africa, while not as good as those of Strachan, point, in so far as they go, to the same low incidence of gastric cancer in the Negro. Vint has reported from Kenya (*4*) and Smith and Elmes from Nigeria (*5*).

These data are not conclusive, but they suggest that there has been a great (possibly a fourfold) increase in the incidence of gastric cancer in the Los Angeles Negroid as an immigrant compared with the African Negroes as sedantes. At the same time there may have been an even greater decrease in the incidence of primary liver cancer since immigration. While there may have been some change in the genes, there has probably been a greater change in environment consequent to migration.

Japanese

The high incidence of gastric cancer (36.2 percent of all malignant tumors) in persons of Japanese ancestry is in striking contrast to the lower incidence in the other racial groups, although this tumor ranked first in all except the Negroids. It also shows a striking resemblance to the incidence reported from Japanese medical centers. Thus, in over 12,000 necropsies at the Pathological Institute of Tokyo Imperial University, Nagayo (*6*) found gastric cancer in first place, where it comprised 42.68 percent of all carcinomas. Similar high necropsy figures are reported from other Japanese cities: 39.2 percent by Ohno from Fukuoka; 35.4 percent by Suzuki from Kyoto; 39.7 percent by Tanaka from Niigata.

Further significance is given in the present data to the outstanding high incidence of gastric cancer by the similarity between the figures for the cancers which are in second, third, fourth, and subsequent places in California and in Japan. These cancers, in Japanese living in both countries, have an incidence which is far below gastric cancer. The evidence, as far as it goes, reveals no difference in incidence of gastric cancer between persons of Japanese ancestry living in California and those living in Japan.

It is possible that insufficient time has lapsed since immigration to have led to changes in gastric cancer incidence even if such were eventually to occur. It is impossible to know from the present data how many of the Japanese were born in Japan and how many were born in California. Among the older people, those in the high-carcinoma age, probably the majority were of the former derivation. If the influences which eventuate in gastric cancer are started at an early age, these persons were not adequate test material for present purposes. The incidence in later generations will be watched with great interest as of greater value for this purpose.

Filipino

Gastric cancer was in fifth place in incidence in a large series of necropsies (13,004) performed by the Department of Pathology and Bacteriology, University of the Philippines (*7*). It was 7.8 percent of all malignant tumors and 0.17 percent of all autopsies. Only 1 case was found in 83 necropsies in Filipinos at Los Angeles. This material is not adequate for statistical analysis or for comparison of incidence in immigrants and sedantes.

Chinese

Data on incidence of gastric cancer in Chinese must be obtained from clinical or surgical material. It is placed in fifteenth position by Hu and Ch'in (*8*) in Peiping, in second place by Braafladt (*9*) in Tsinan, and in seventh place by Bercovitz (*10*) in Hainan. In replies to a questionnaire by Maxwell (*11*) two hospitals placed gastric cancer first, nine placed it second, and three placed it third. No case was found in the present series at Los Angeles, but it would be unwise to make, from this, a comparison in immigrants and sedantes.

SUMMARY

A comparison of the incidence of gastric cancer found in 35,293 necropsies at the Los Angeles County Hospital in the 3 great races of mankind, namely Caucasoid, Negroid, and Mongoloid (separate analyses for Japanese, Chinese, and Filipinos), and in Mexicans, revealed the following: (1) The incidence was slightly but significantly lower in Negroids than in Caucasoids. This was due to a lower incidence in the Negroid male. (2) The incidence was about 3 times greater in Japanese than in Caucasoids. (3) The incidence in Mexicans was not significantly different from that in Caucasoids. (4) The data for Chinese and Filipinos were inadequate for establishing comparative incidences.

Comparison of the incidence of gastric cancer in persons of the different races considered as immigrants with the corresponding racial sedantes revealed the following: (1) It was impossible to determine from the available data whether incidence in the Caucasoids had changed since they immigrated from Europe. (2) Data from Mexico with which comparisons can be made with the Los Angeles material are not at hand. (3) It is probable that the Los Angeles Negro has a higher incidence than the African Negro. (4) The Chinese and Filipino materials were inadequate for comparisons of immigrants and sedantes.

CONCLUSIONS

The data obtained from comparing Negroids as immigrants and sedantes strongly suggest that an environmental factor or factors in part determine the occurrence of gastric cancer. Corresponding data for the other races (Caucasoids, Mexicans, Japanese, Chinese, and Filipinos) shed no light on this point either because the number of cases was insufficient or because adequate data on the sedantes are lacking.

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Chairman Brunschwig: The next group of seven papers will be taken together with discussion at the end. The first one is by Dr. H. Davis Chipps of Seattle, Washington, The Washington State Tumor Registry and its Role in Problems Regarding Gastric Cancer and Cancer Research.

THE WASHINGTON STATE TUMOR REGISTRY AND ITS ROLE IN PROBLEMS REGARDING GASTRIC CANCER AND CANCER RESEARCH [1]

By H. Davis Chipps, M. D., *Department of Pathology, University of Washington School of Medicine, Seattle, Wash.*

The purpose of this paper is to describe the recently organized Washington State Tumor Registry and to indicate that its function is not only for statistical purposes but that its diagnostic service, teaching facilities, and research potentialities are even more important. Its application to such special problems as gastric cancer will be pointed out.

In order to insure that all of these features be developed properly, the registry was established jointly by: (1) the State Society of Pathologists representing the State Medical Association; (2) the State Department of Health, which is responsible for financing and administering the program; and (3) the Department of Pathology in the Medical School, which is professionally responsible for the diagnosis, teaching, and many of its research applications.

The mechanism for administering the program is simple. The pathologist submits to the registry a copy of his report together with one or more tissue sections for which he is reimbursed 50 cents per case. This is entirely on a voluntary basis, and no material is accepted except that submitted by pathologists. The sections are reviewed by members of the Department of Pathology, and a report of this review is sent directly to the contributing pathologist, not to the physician caring for the case, in order to avoid confusion or embarrassment. If more material is desired for special studies, it is obtained from the pathologist. In the event of differences of opinion the case is referred to the Armed Forces Institute of Pathology. Material from rare, interesting, or controversial cases is obtained, and sets of slides are prepared in the registry laboratory. Four times a year a seminar is held for the State Society of Pathologists. In addition, weekly seminars are held for all interested individuals, such as students, research associates, and visiting pathologists.

When the pathologist submits his original diagnosis to the physician, he encloses a clinical record form with the request that the physician fill in the data and mail it directly to the registry. It is of interest that in our first 3,000 accessions of malignant neoplasms that clinical data has been voluntarily sent in on 85 percent of them. By comparison, the reporting of syphilis is mandatory in the State, and it is estimated that not more than 80 percent of cases are being reported.

[1] Presented at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

With reference to the clinical data form, it has been kept as simple as is consistent with securing pertinent information, although some detail in reference to treatment is desirable. Once a year a follow-up is obtained on each case, for this is considered to be essential in checking errors in diagnosis and the general effects of therapy.

A compilation of the first 3,000 registered cases of malignant neoplasms is shown in table 1 and is compared with figures derived by two other different techniques. The first of these is a direct survey by Dorn, and the second is the compulsory reporting method used in New York State. It can be seen by comparing the three columns that they are essentially similar except in two categories where our data varies significantly from the other two. There is a lower percentage of cancer of the stomach and a higher incidence of skin neoplasms.

TABLE 1.—*Percentage distribution of malignant tumors*

	Male			Female		
	USPHS Dorn	New York State [1]	Washington	USPHS Dorn	New York State [1]	Washington
Buccal cavity and pharynx	10.0	7.0	7.6	2.2	1.5	2.1
Digestive organs and peritoneum	36.6	32.1	[2] 25.8	21.5	23.3	[2] 15.4
Esophagus	-------	2.0	2.8	-------	.5	.4
Stomach	-------	10.6	[2] 7.2	-------	5.5	[2] 2.6
Intestines	-------	7.9	8.2	-------	8.2	7.6
Rectum and anus	-------	6.9	5.1	-------	4.8	3.6
Liver and biliary	-------	2.2	1.6	-------	2.6	.9
Pancreas	-------	2.1	.9	-------	1.2	.3
Respiratory system	8.4	8.4	7.8	1.5	1.3	1.4
Uterus	-------	-------	-------	22.0	18.4	20.9
Other female genital organs	-------	-------	-------	5.7	5.4	7.3
Breast	-------	.3	.8	23.9	23.9	25.3
Male genital organs	10.7	11.5	8.9	-------	-------	-------
Urinary organs	7.3	6.8	7.9	3.3	2.6	2.7
Skin	17.3	15.0	[2] 25.2	11.3	8.4	[2] 12.7
Brain and central nervous system	-------	2.1	1.3	-------	1.0	1.1
Other and unspecified	-------	9.8	12.3	-------	9.0	10.3
Tumors, unspecified	-------	3.7	-------	-------	2.9	-------
Leukemia	-------	2.1	.9	-------	1.5	.4
Hodgkin's disease	-------	1.2	1.3	-------	.8	.4

[1] 1944 data.
[2] Statistically significant variation.

The relatively small number of cases of gastric carcinoma reported has provoked interest. Since the death rate based on vital statistics from stomach cancer is no lower than the usual experience, our low incidence of cases reported to the registry apparently constitutes an artefact. That there is a strong element of selection seems likely from the fact that cancer of other parts of the gastrointestinal tract are reported with expected relative frequency. Since almost 90 percent of diagnosis in the registry are based upon biopsy or operative findings, it is very likely that stomach cancers are diagnosed clinically or radiologically too late to justify exploratory surgery or resection. Such study of registry figures may very well

point up possible areas for additional research and professional educational activities.

As an example of the way in which the registry will serve as a basis for certain research problems, the current plans with reference to gastric carcinoma alone will be cited. These fall into three categories. In the first, the radiologists will be asked to cooperate in a special study of gastric cancer cases by registering their diagnoses of suspected gastric malignancies. Many of these cases may never get to pathologists. Follow-up of these cases should assist in establishing the validity of diagnosis. In the second phase, which will again be a problem in clinical research, a program is to be developed involving the roentgen findings in apparently normal persons. These individuals will be followed by further examination at yearly intervals. New accessions will be checked against these records, therefore, providing a follow-up control on the experimental group. The third problem will form part of a program in cooperation with the Cancer Control Division of the National Cancer Institute in an attempt to reevaluate certain of the tests previously claimed or currently being developed for the detection of cancer. Specimens of blood for the control group, which will constitute 100,000 individuals over the next estimated 5 years, will be obtained from the King County Central Blood Bank and recorded in the registry against the future possibility of the development of cancer in these individuals. Actually, sera are already being stored in deep freeze units for current testing and for availability in future years. The same holds true for individuals with known cancer, and these come from 12 hospitals in which it is possible to get detailed laboratory data.

This program will result, over a period of years, in a thorough evaluation of any possible significant tests which heretofore have not been systematically explored. Finally, such a program is serving as a basis for extensive original investigation in the field of immunochemistry and in certain phases of protein chemistry.

SUMMARY

It is felt that this program serves more than a statistical need since it utilizes the material for diagnostic problems that confront the pathologists in the various communities; since it serves as a medium for teaching fellows, interns, residents, and other interested individuals; since it serves as a mechanism by which the relative effectiveness of various forms of therapy in different types of neoplasms can be checked; and finally, since all of these cases constitute a nucleus from which serial studies of body secretions and tissues can be obtained for original as well as evaluation studies of previous and projected tests for cancer.

Chairman Brunschwig: The next paper will be read by Dr. David State, Minneapolis. It is entitled An Attempt to Identify Likely Precursor Groups of Gastric Cancer.

AN ATTEMPT TO IDENTIFY LIKELY PRECURSOR GROUPS OF GASTRIC CANCER [1] [2]

By David State, M. D., David Gaviser, M. D., Thomas B. Hubbard, Jr., M. D., and Owen H. Wangensteen, M. D., *Department of Surgery, University of Minnesota Medical School, Minneapolis, Minn.*

A cooperative clinical study embracing the Departments of Pathology, Radiology, Medicine, and Surgery was begun at the University of Minnesota Hospitals in July of 1945, in an effort to uncover early silent gastric carcinoma. At the Gastric Cancer Conference held in Chicago in 1946 (*1*), our initial experiences with this study to determine likely precursors of gastric cancer were reported. We felt the preliminary results of this study were sufficiently encouraging to warrant its continuation. In this presentation our findings tc date will be reported.

MATERIAL AND METHOD

The following groups were considered to be potential harborers of silent gastric carcinoma and were set aside for special study. The patients, outpatient clinic registrants, at the University Hospitals, were past the age of 50 years and were free of digestive complaints.

Group I: Patients with achlorhydria and hypochlorhydria: Three successive doses of 0.5 mg. of histamine[3] at 20-minute intervals were used to stimulate the flow of gastric acid maximally. Free hydrochloric acid was tested for with Topfer's reagent and phenolphthalein as indicators. Patients with achlorhydria were given gastric roentgenograms. These examinations consisted of fluoroscopic observations after the ingestion of barium; an appropriate number of routine and spot films were taken for permanent record and for facilitating the diagnosis of obscure lesions. In a number of instances, usually because of reactions, patients were not given the three conventional doses of histamine. These patients have been designated separately depending upon the number of histamine injections given.

In 1940 Wetherby (*2*), of our institution, reported upon a group of individuals who were observed to be achlorhydric after a single injection of 0.5 mg. of histamine. As many of these patients as possible were recalled for repeat gastric analyses and roentgenograms of the stomach.

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[1] Read by Dr. David State at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] The researches presented here were supported by the following grants: National Cancer Institute, National Institutes of Health, U. S. Public Health Research Grant, The Flora L. Rosenblatt Fund for Cancer Research, and the Malignant Disease Research Fund of the University of Minnesota.

[3] Histamine diphosphate was used in this study.

A number of patients had free hydrochloric acid after histamine stimulation but in reduced amounts. If the maximum amount of free hydrochloric acid did not exceed 30 degrees, patients exhibiting this finding were designated as hypochlorhydrics and also received gastric roentgenograms.

Group II: Patients with pernicious anemia: This group included previously observed as well as new patients with pernicious anemia. The diagnosis in each case was confirmed by bone marrow studies. The patients had gastric analyses, gastric roentgenograms, and, whenever possible, gastroscopic examinations. Many of the previously observed patients with pernicious anemia had been subjected to frequent gastric roentgenograms, for these patients had been studied carefully by Drs. Rigler, Kaplan, and Fink (*3*).

Group III: Individuals with a positive family history of gastric carcinoma: The living relatives of patients who had been operated upon at the University Hospital for gastric carcinoma were requested to report for gastric analyses as well as gastric roentgenograms. In this group, although gastric analyses were made routinely, all received gastric roentgenograms regardless of whether the patient showed achlorhydria, hypochlorhydria, or normal gastric acidity.

Group IV: Patients with occult blood in the stool and/or hemoglobin values of 11.0 gm. or less: From July 1, 1946, these individuals were also studied in an effort to include more individuals potentially harboring gastric cancer. The method used for testing for occult blood was the guaiac reaction. Whenever possible, the patient was placed on a meat-free diet for 3 days prior to the test. These patients received gastric roentgenograms regardless of the degrees of free hydrochloric acid observed on gastric analyses.

Observations: See tables 1 and 2.

TABLE 1.—*Incidence of achlorhydria and hypochlorhydria in patients of age groups eligible for precursor gastric cancer study July 1, 1945, to November 1, 1948*

Out-patients registered, number	Gastric expression		Achlorhydrics (histamine)									Hypochlorhydrics		
			Triple			Double			Single					
	Number	Sex	Number	Sex	G. I.	Number	Sex	G. I.	Number	Sex	G. I.	Number	Sex	G. I.
4,048	1, 378	F	183	F	----	104	F	----	129	F	----	67	F	----
	2, 038	M	342	M	----	139	M	----	152	M	----	85	M	----
Total	3, 416	----	525	----	836	243	----	313	281	----	232	152	----	93

TABLE 2.—*Gastric cancer precursor study, July 1, 1945, to November 1, 1948*

Individuals		G. I. series	Findings	
Type	Number		Polyps	Cancer
Achlorhydrics	1,049	1,381	[1] 27	[2] 7
Hypochlorhydrics	152	93	1	
Wetherby achlorhydrics	110	165	[3] 6	1
Pernicious anemia patients:				
48 new	92	186	[3] 4	3
44 old				
Relatives of patients with gastric cancer	75	75		
Patients with hemoglobin level of 11 gm. or less	54	54		
Precursors with occult blood in feces	46	34		
Total	1,578	1,988	38	11

[1] Plus 14 questionable.
[2] 2 additional carcinomas missed through organizational errors; 1 additional carcinoma missed through misinterpretation of X-rays.
[3] Plus 1 questionable.

From July 1, 1945, to November 1, 1948, there were 4,048 outpatient registrations in the age group that was studied. Gastric analyses were done on 3,416 individuals (1,378 females and 2,038 males). Five hundred and twenty-five people were achlorhydric after 3 successive doses of histamine (183 females and 342 males), and in this group 836 gastrointestinal X-ray examinations were obtained; 243 patients were achlorhydric to 2 doses of histamine (104 females and 139 males), and in this group 313 gastrointestinal X-ray examinations were obtained; 281 individuals were achlorhydric to a single dose of histamine (129 females and 152 males), and in this group 232 gastrointestinal X-ray examinations were obtained. Additionally, there were 152 hypochlorhydrics (67 females and 85 males), and in this group 93 gastrointestinal X-ray examinations were obtained. In the Wetherby group of patients of 1940, there were 110 individuals who were achlorhydric, and 165 gastrointestinal X-ray examinations were obtained. Thus, in the achlorhydrics and hypochlorhydrics there were 1,311 individuals studied, and 1,639 gastrointestinal X-ray examinations were obtained. In this group of achlorhydrics and hypochlorhydrics, 8 silent gastric carcinomas and 34 gastric polyps were found.

Of the eight gastric carcinomas, five were early, well localized, and without metastases; the remaining three were farther advanced and had lymph-node metastases. Two additional patients with carcinoma were missed through organizational errors, and one other patient was missed because the gastric roentgenograms were misinterpreted, although on reexamination of the films the presence of a lesion was identified. This carcinoma was recognized at the time of an operation performed on this patient for another unrelated condition.

There were 92 patients with pernicious anemia, of whom 48 were new and 44 had been observed here prior to the commencement of this

study. In this series 186 gastrointestinal X-ray examinations were obtained. Three gastric carcinomas and 4 polyps were found.

In 75 relatives of patients with gastric cancer, on whom 75 gastrointestinal X-ray examinations were obtained, no gastric polyps or carcinomas were found.

In 54 patients with hemoglobin levels below 11.0 gm, 54 gastrointestinal X-ray examinations were obtained. Forty-six patients with occult blood in the stools had 34 gastrointestinal X-ray examinations, and no polyps or gastric carcinomas were found.

DISCUSSION

Achlorhydria: The frequent association of achlorhydria with gastric carcinoma suggests the validity of the acceptance of the criterion of achlorhydria in surveys aimed at the detection of gastric cancer. Hebbel and Gaviser (*4*) found that about 65 percent of the patients with gastric carcinoma have histamine achlorhydria, and an additional 21 percent show hypochlorhydria (less than 30 degrees free acid). In contrast to the high incidence of achlorhydria and hypochlorhydria seen with gastric cancer we (*5*) noted that achlorhydria and hypochlorhydria are not consistently seen with extragastric malignancies, for in this group the incidence of achlorhydria and hypochlorhydria is about 30 percent, a circumstance which appears to be the same for individuals of the same age group who do not have carcinoma.

The studies of St. John, Swenson, and Harvey (*6*) and Dailey and Miller (*7*) indicated the need for some practical plan for filtering off those cases most likely to harbor a silent gastric carcinoma. The former group of investigators subjected 2,413 patients over the age of 50 without gastric complaints to a brief fluoroscopic examination of the stomach after the injection of barium. In this group of patients 2 silent gastric cancers and 1 lymphosarcoma were found. No gastric polyps were encountered. Dailey and Miller (*7*) did gastric roentgenograms on 500 presumably normal men over the age of 50 free of digestive complaints. Three gastric lesions were found, 1 benign gastric ulcer, 1 polyp, and 1 antral gastritis. In this precursor gastric cancer study the use of achlorhydria and hypochlorhydria has permitted the discovery of a greater number of unsuspected gastric carcinomas for a given number of X-ray examinations of the stomach. Out of 1,546 gastrointestinal X-ray examinations in 1,159 achlorhydric patients and 93 gastrointestinal X-ray examinations in 152 hypochlorhydric patients, 8 silent gastric cancers and 34 gastric polyps were found (table 3).

Pernicious anemia: The relationship of pernicious anemia and gastric carcinoma has been noted by a number of observers (Rhoads (*8*), Borrmann (*9*), and Rigler (*10*)). In our previous report, including 79 patients with pernicious anemia, no gastric carcinomas or polyps were found. We felt this was due to the fact that we were examining a very select group in that they represented individuals who had undergone careful scrutiny

TABLE 3.—*Studies for determining unsuspected gastric cancer*

Investigators	Number of individuals having X-ray examinations of the stomach	Type of individuals studied	Findings
St. John, Swenson, and Harvey (New York, 1944).	2, 413	Presumably healthy individuals over the age of 50 free from digestive complaints.	2 gastric carcinomas. 1 lymphosarcoma.
Dailey and Miller (San Francisco, 1945).	500	Presumably healthy males over 45 years presumably free of digestive complaints.	1 benign gastric ulcer. 1 polyp. 1 antral gastritis.
Present Study (Minneapolis, 1945–1948).	1, 411	Outpatient clinic registrants over the age of 50, free of digestive complaints and with histamine achlorhydria or hypochlorhydria.	8 gastric carcinomas. 34 polyps.

2 additional carcinomas missed through organizational errors.
1 additional carcinoma missed through misinterpretation of X-rays.

just a short time previously by Dr. Rigler and his coworkers. The discovery of 3 gastric cancers and 4 polyps in 92 patients with pernicious anemia, since our initial report, would tend to confirm the suggestion that a greater than normal incidence of gastric neoplasms is to be anticipated in patients who have pernicious anemia. Also, it stresses the need for frequent gastric roentgenograms in patients with pernicious anemia.

Individuals with a family history of gastric carcinoma: In our initial report (*1*) we noted that the incidence of gastric cancer was 15.6 percent in the families of patients with gastric carcinoma, whereas, in the families of patients with gallbladder disease, the incidence of gastric carcinoma was 10 percent; in patients with colonic cancer, it was 9.4 percent, and in the families of patients with hemorrhoids, the incidence of gastric carcinoma was 12 percent. This increased incidence of gastric malignancies in the families of patients with gastric carcinomas was, however, not statistically significant. It was our feeling, however, that relatives of patients with gastric carcinoma should be studied. Responses to our requests were extremely poor. We contacted over 200 individuals, but only 75 appeared for examination. In this group of 75, 75 gastrointestinal X-ray examinations were obtained, and no gastric carcinoma or polyps were found. Because of the poor response in this group we have decided to abandon this portion of the study for the present.

Patients with hemoglobin level below 11.0 gm. and patients with occult blood in the stool: The number of individuals in these 2 categories is small, 54 and 46 respectively, and no gastric neoplasms were found. A large number of patients in the study had low hemoglobin values and/or occult

blood in the stools, but definite causes for these abnormalities were noted. In addition, a number of these individuals also had achlorhydria or hypochlorhydria and consequently were placed in these categories. The individuals that appear on our records in these groups represent those who do not fall into the other categories in the study.

Gastric Polyps: Thirty-eight gastric polyps have been uncovered in this study. Although many authors (Bormann (*9*) and Rigler (*10*)) have indicated polyps of the stomach as precancerous lesions, their actual fate is difficult to evaluate in any given instance as Spriggs and Marxer (*11*) have indicated. It has been one of the purposes of this study to attempt to secure a more satisfactory answer to the problem of whether or not gastric polyps do become malignant. To this end, in addition to the 38 polyps discovered in this study, the case records of 47 additional patients have been culled from the hospital records from 1936 to the present time (table 4). In each instance the diagnosis of polyps has been made only after roentgenographic or gastroscopic examinations or by employment of both methods.

TABLE 4.—*Gastric polyps*

Patients studied	Polyps	Patients operated on	Malignancies
	Number	*Number*	*Number*
Gastric cancer precursor group, July 1, 1945, to December 1, 1948	38	11	3
Hospital records, July 1, 1936, to December 1, 1948	47	20	4
Total	85	31	7

To date, 31 of the 85 patients with gastric polyps have been operated upon. In 7 instances the polyps were found to be malignant by histological examination. In the group of 38 polyps discovered in this precursor gastric study, 11 patients have been operated upon. In 3 instances the polyps removed were diagnosed as adenocarcinoma, and in 1 instance, there were metastases to the regional lymph nodes. There is a question whether the malignant polyps which were discovered had arisen from previous benign polyps or whether they were malignant from their onset. Although no definite answer can be given to the problem of malignant degeneration of benign polyps, it is important to notice that of 85 cases with polyps 7 were malignant, an incidence of approximately 8 percent.

Further studies are being carried out determining the criteria which indicate the presence of malignancy, and efforts are being made to determine the eventual fate of all gastric polyps under our observation.

CANCER DETECTION CENTER

Largely as a result of the stimulation given to the problem of uncovering early, silent carcinomas in the precursor gastric cancer study, the Cancer Detection Center was set up at the University of Minnesota in March, 1948.

Persons examined at the Center are apparently in good health and over the age of 45. Before coming to the Cancer Detection Center, the patients are placed on a meat-free diet for 3 days and are requested to bring in a stool specimen which is examined for occult blood both by the benzidine and guaiac techniques. In addition to the routine physical examination, all individuals are given complete blood and urine examinations, a routine proctoscopic examination, and a gastric analysis, using a single dose of ½ mg. of histamine as a gastric secretion stimulant. Females are given a pelvic examination by a gynecologist, and recently smears, stained by the Papanicolaou technique, have been taken of vaginal and cervical areas. A photofluorogram of the chest is taken on everyone, and if there are any abnormal findings, a routine chest plate is obtained.

In an effort to interpose practical filters, the following individuals receive gastric roentgenograms:

(1) Patients with achlorhydria or hypochlorhydria, less than 30 degrees free acid;

(2) patients with occult blood in the stool;

(3) patients with unexplained hemoglobin levels below 11.0 gm.;

(4) patients with a strong family history of gastric cancer.

The following individuals receive barium enemas:

(1) Patients with occult blood in the stool;

(2) patients with abnormal proctoscopic findings, such as polyps, bloody mucus, etc.;

(3) patients with unexplained hemoglobin levels below 11.0 gm.;

(4) patients with a family history of colonic cancer.

FINDINGS

From March 1, 1948, to November 31, 1948, 1,143 patients (525 males and 618 females) were examined. In this group, 15 silent carcinomas have been found. The types of lesions and organs involved are given in table 5. From table 6 it can be seen that of the 1,143 patients, 201 were achlorhydric and 123 were hypochlohydric to histamine. In this group 289 gastrointestinal X-ray examinations were obtained, and 1 patient with gastric carcinoma and 1 with a gastric polyposis were found. Two additional patients with gastric carcinoma were found, but on critical review of the record it was felt that these patients had symptoms which, although mild, were sufficiently definite to exclude considering their malignancies as being silent. The one asymptomatic carcinoma that was found was less than 1.0 cm. in diameter and was confined to the gastric mucosa.

TABLE 5.—*Summary of findings at the Cancer Detection Center, University of Minnesota Hospitals, from March 1 to November 30, 1948, inclusive*

Finding	Number
Carcinomas:	
Basal-cell carcinoma	4
Cancer of rectum	5
Cancer of cecum	2
Cancer of esophagus	1
Cancer of stomach	3
Hyperkeratosis of skin with cancer in situ	1
Lymphosarcoma, involving bone marrow	1
Total	17
Precancerous lesions:	
Gastric polyposis	1
Keratosis, skin	21
Kraurosis vulvae	5
Leukoplakia, oral	23
Leukoplakia, labia majora	3
Leukoplakia, larynx	1
Leukoplakia, portio-cervix	1
Pigmented naevi in areas of irritation	91
Polyp of vocal cord	1
Rectal polyps, including adenomata	122
Thyroid adenomas	17
Total	286
Suspicious tumors requiring further diagnostic procedures:	
Adnexal masses	10
Cystic right ovarian mass	1
Nodules, axilla	1
Nodules, maxilla	1
Nodules, groin	1
Nodules, breast	10
Nodules, thigh	1
Nodular area, right side post lip of cervix	2
Preauricular mass	1
Questionable mass LUQ	1
Extra rectal mass	1
Tumor, chest	1
Tumor, left parotid	1
Tumor, lip	7
Tumor, neck	1
Tumor, nose	1
Tumor, tongue	7
Tumor, tonsillar pillar	2
Ulcer of cervix	3
Uterine mass	3
Total	55
Total number of patients	1,143

TABLE 6.—*Incidence of achlorhydria and hypochlorhydria at the Cancer Detection Center, March 1 to December 1, 1948*

Total number of patients	1,143
Patients with histamine achlorhydria or hypochlorhydria:	
Achlorhydria	201
Hypochlorhydria	123
Total	324
X-rays (G. I. series) on these groups:	
Achlorhydria	193
Hypochlorhydria	96
Total	289
Total number of gastric cancers	3
Total number of gastric polyps	1

In addition to the malignancies noted, 286 precancerous lesions have been discovered. Among these were 122 rectal polyps. The incidence of rectal polyps in this group of individuals over the age of 45 was thus approximately 10 percent. This figure is somewhat higher than previous reports in the literature would indicate, although Swinton (12) reported an incidence of rectal polyps of 7 percent. It must be remembered, however, that the age group which we are examining is a select one; namely, over the age of 45 and, consequently, it might be expected that the number of rectal polyps would be greater in this group than in the younger age groups. We found in 55 instances (table 5) lesions such as pelvic masses, tumors of the breast, etc., which required further diagnostic studies to determine their exact nature; these individuals were sent back to their referring physician to have the appropriate diagnostic procedures carried out. A number of these lesions undoubtedly will be malignant and will further increase the number of malignancies uncovered at the Cancer Detection Center.

Considerable controversy exists at the present time concerning the practicability and value of a cancer detection center. The pilot plant which we have set up at the University Hospital has as its prime purpose the evaluation of some of these points. Our center, we feel, differs from others in that in addition to the routine physical examination, as well as blood and urine examinations, the patient has a proctoscopic examination, a gastric analysis, and an effort is made to determine whether occult blood is present in the stools.

If achlorhydria and hypochlorhydria to histamine and the presence of occult blood in the stool prove to be worth-while preliminary tests to suggest the necessity for further roentgen studies of the gastrointestinal tract, particularly of the stomach and the colon, it is apparent that these are tests which may be done in any physician's office who affects an interest

in cancer. The devising of such filters, in the absence of specific biologic tests for cancer, will help to lessen the burden of X-ray studies which fall heavily on roentgenologists in the Cancer Detection Centers.

SUMMARY AND CONCLUSION

In an effort to uncover early silent gastric carcinomas and determine what conditions are most likely to favor the development of gastric cancer, the following clinical groups have been scrutinized and considered as possible precursors of gastric cancer. Individuals in these groups have been studied by means of gastric roentgenograms and in some instances with gastroscopy:

(1) Patients over the age of 50 with histamine achlorhydria and hypochlorhydria;
(2) patients with pernicious anemia;
(3) patients with unexplained hemoglobin level below 11.0 gm.;
(4) patients with occult blood in the stools;
(5) relatives of patients with gastric carcinoma.

From July 1, 1945, to November 1, 1948, 3,416 individuals meeting the requirements had gastric analyses: 525 were found to be achlorhydric to 3 successive doses of 0.5 mg. of histamine; 243 were achlorhydric to 2 consecutive doses of histamine; and 281 were achlorhydric to a single .05 mg.-dose of histamine. An additional 152 patients were found to be hypochlorhydric, that is, they had less than 30 degrees of free acid in their gastric juice. In this group 1,474 gastrointestinal X-ray examinations were obtained. Seven silent carcinomas and 28 gastric polyps were found. In addition, 2 gastric carcinomas were missed through organizational errors and 1 through misinterpretation of the gastric roentgenogram.

In a group of 110 achlorhydric individuals obtained from a previous study by Wetherby, 165 roentgenograms were obtained. One gastric carcinoma and 6 gastric polyps were found in this group.

Ninety-two patients with pernicious anemia had 186 gastrointestinal X-ray examinations. Three gastric carcinomas and 4 gastric polyps were found.

Seventy-five relatives of patients with gastric carcinomas, 54 patients with unexplained hemoglobin levels below 11.0 gm., and 46 patients with occult blood in the stools were submitted to X-ray examinations. No gastric neoplasms were discovered in this miscellaneous group of 163 patients.

Thirty-eight gastric polyps were uncovered in this precursor gastric cancer study. In 11 instances the patients were operated upon, and in this group 3 adenocarcinomas were discovered. An additional 47 cases were culled from the hospital records and placed under observation. In this group 20 patients were operated upon, and 4 malignancies were found. The incidence of malignant polyps in this group of 85 patients with gastric polyps under observation is thus approximately 8 percent.

In the Cancer Detection Center 1,143 patients over the age of 45 have been examined. In this group 15 silent carcinomas have been found, of

which 1 was a gastric carcinoma. One hundred twenty-two rectal polyps have been uncovered, an incidence of approximately 10 percent.

It is our belief that the results obtained in both the gastric cancer precursor study as well as the cancer detection center suggest that it is both possible and practical to survey large groups of individuals in an effort to uncover early silent malignancies.

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Chairman Brunschwig: The next paper, listed for Dr. Russell H. Morgan, will be given by Dr. John Roach of Baltimore; it is entitled Photofluorograph in the Detection of Gastric Cancer.

PHOTOFLUOROGRAPHY IN THE DETECTION OF GASTRIC CANCER [1] [2]

By JOHN F. ROACH, ROBERT D. SLOAN, and RUSSELL H. MORGAN, *Department of Radiology, Johns Hopkins University and Hospital, Baltimore*

Approximately 10 months ago a photofluorographic method of examining the stomach in an attempt to detect the early presence of a carcinoma was begun at the Johns Hopkins Hospital. The statistical background and the instrumentation necessary for this study have been described in some detail in previous publications.[3] It is our purpose at this time to present our results in the first 2,500 cases.

Table 1 shows the age, race, and sex distribution of the study. It will be noted that the vast majority of the patients were 40 or more years of age. They were selected on this basis since carcinoma of the stomach is quite rare prior to that age. We did, however, examine 323 patients below 40 since we wished to become familiar with the photofluorographic appearance of the stomach in all age groups. It will be seen in table 2 that 94 percent of the examinations were satisfactory for diagnostic purposes, and of these, 86.9 percent were normal and 7.1 percent abnormal. There were 150, or 6 percent, that were unsatisfactory.

TABLE 1.—*Photofluorographic examinations by age, race, and sex*

Age	WM	CM	WF	CF	Total	Percent
10–39	119	9	181	14	323	12. 9
40–89	760	391	728	298	2, 177	87. 1
Total	879	400	909	312	2, 500	100
Percent	35. 1	16. 0	36. 3	12. 5		

TABLE 2.—*Photofluorographic examinations of the stomach*

Condition	Normal		Abnormal		Total	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Satisfactory for diagnosis	2, 172	86. 9	178	7. 1	2, 350	94. 0
Unsatisfactory for diagnosis					150	6. 0
Total					2, 500	

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] This investigation was supported by a grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

[3] ROACH, J. F., SLOAN, R. D., and MORGAN, R. H.: The detection of gastric carcinoma by photofluorographic methods. Am. J. Roentgenol. & Radium Therapy 61: 183-187, 187-194 (1949).

Table 3 illustrates the three main reasons for the unsatisfactory films. The first group consists of technical failures, including poor positioning and mechanical and electrical breakdown. This consitutes 3 percent of the total and should be reducible to almost zero as more experience is gained in the method. The second cause of difficulty was the presence of excessive quantities of food within the stomach of the patient at the time of examination. This amounted to only 2 percent of the cases and seems to us to be an insignificantly low figure, since no real effort was made to have the patients in a fasting state at the time the examinations were conducted. The third reason for unsatifsactory films was the marked obesity of 23 patients; these individuals were too large to obtain films of good quality either photofluorographically or by the standard roentgen methods. This, too, is a very small group and amounts to only 1 percent.

TABLE 3.—*Unsatisfactory examinations*

Cause	Number	Percent
Technical failures (poor positioning, etc.)	76	3.0
Excessive food within the stomach	51	2.0
Marked obesity of the patient	23	1.0
Total	150	6.0

We adopted the customary gastrointestinal fluoroscopic and radiographic examination as a standard of comparison for this study, since it is an accepted fact that the roentgenological method is probably the most reliable means available for the diagnosis of carcinoma of the stomach. Thus, all patients who demonstrated any abnormality or a suspicion of abnormality in their photofluorographic examinations were asked to return for regular radiological studies. A total of 280 patients had such a follow-up, and table 4 shows the correlation of these examinations with the photofluorographic.

TABLE 4.—*Correlation between gastric findings of regular and miniature gastrointestinal series*

Findings	Number	Percent
Normal on both miniature and regular	172	61.5
Normal on miniature but abnormal on regular	6	2.1
Abnormal on both miniature and regular	40	14.2
Abnormal on miniature but normal on regular	62	22.2
Total	280	100.0

There were 40 patients who were abnormal on both the photofluorographic and the regular G. I. studies. The following tabulation shows the pathological diagnosis of these patients. It will be noted that there were 13 cases of proven gastric malignancy and 5 who showed all roentgen

manifestations of a stomach cancer but as yet have not been microscopically proven. The remaining 22 cases were divided among the usual benign states.

PATHOLOGY DEMONSTRATED ON MINIATURE AND CONFIRMED BY REGULAR GASTROINTESTINAL STUDIES

Gastric malignancy, microscopically proven	13
Gastric malignancy suspected but not yet proven	5
Benign lesions simulating malignancy preoperatively	2
Gastric ulcer	5
Hiatus hernia	11
Gastric diverticula	2
Subtotal gastric resection	1
Gastroenterostomy	1
Total	40

Table 4 also shows that a number of patients who were said to be normal on the miniature films were also examined by standard methods. This was done to check the negative photofluorographic patients, and there were 172 such individuals. Six patients who were said to be normal on the miniature films were found to be abnormal on the regular. On the surface this may appear to be a serious error; however, none of these 6 patients had a malignant lesion. All were relatively insignificant benign processes. There were 62 patients that were classified as abnormal on the miniature films but were found to be normal on the regular G. I. study. At first glance, this too seems to be quite a discrepancy, but it is one that we fully expected, since at the start of this study we decided that any patient who appeared abnormal or suspiciously so would be considered abnormal until proven otherwise. We felt justified in doing this since at the beginning of this project we were totally inexperienced with the method and felt that this was a valuable assurance to avoid the missing of positive cases. As the study has progressed and we have gained experience in the photofluorographic manifestations of gastric carcinoma, the number of patients shown to be abnormal on miniature studies but normal on the regular has been greatly reduced.

Table 5 is a tabulation of the 18 patients with proven or suspected gastric carcinoma. It will be noted that of these, 15 had symptoms which could be related to their gastric disease. In most instances these symptoms were quite prominent. In a few cases they were very mild and in 2, scarcely detectable. There were 3 patients who were totally devoid of any complaints or physical findings which might suggest the presence of a carcinoma. Three asymptomatic cases are statistically the number [4] which should be expected in the examination of 2,500 normal individuals, and it thus appears that the photofluorographic method is at least a reasonably accurate method for the detection of early gastric cancer. The attitude which the 3 asymptomatic patients have taken toward the news that they have gastric cancer has been most interesting and some-

[4] DORN, H. F.: Illness from cancer in the United States. Pub. Health Rep. 59: 33-48, 65-77, 97-115 (1944)

what distressing. It seems impossible for the symptom-free person to believe that he can have a cancer. Accordingly, we have been unable to convince any of these people that he is in need of surgical attention. From the viewpoint of our study, this is quite unfortunate since we were hopeful of removing these malignant growths while in a curable stage. However, this situation has given us the opportunity to gain some knowledge of the silent period of gastric carcinoma. We are reexamining these patients at frequent intervals, and we find that the lesions are slowly increasing in size. As yet, however, none of these patients has developed any gastric complaint. We are sure that when they do, they will then be readily amenable to surgery, but certainly their curability will be appreciably reduced. The gastroenterologist at our clinic has just recently acquired a new flexible gastroscope with which it is possible to take biopsies, and it is hoped that with this additional tool and the evidence which it will provide we will convince our asymptomatic patients that early operation is desirable.

TABLE 5.—*Tabulation of 18 patients with proven or suspected gastric carcinoma*

No.	Age	Race	Sex	Diagnosis	Symptoms
59	45	C	M	Scirrhous carcinoma	Moderate.
99	50	C	M	Papillary adenocarcinoma	Do.
588	56	W	F	Mucous-forming adenocarcinoma	Do.
1, 166	50	C	M	Papillary adenocarcinoma	Do.
1, 196	51	C	F	Adenocarcinoma	Do.
1, 318	69	C	M	do	Do.
1, 484	74	C	M	Mucous-forming adenocarcinoma	Marked.
1, 508	78	C	M	Adenocarcinoma	Do.
1, 374	53	C	M	Neurogenic sarcoma	Do.
1, 836	60	C	M	Adenocarcinoma	Do.
1, 756	50	C	F	do	Mild.
1, 288	69	W	M	Carcinoma	Minimal.
538	54	C	F	Adenocarcinoma	Mild.
846	74	C	F	Not yet established	Moderate.
891	53	C	F	do	Minimal.
1, 044	60	C	F	do	Absent.
2, 118	78	W	F	do	Do.
2, 193	68	C	M	do	Do.

In the group of symptomatic patients who have been operated upon, most cases were resectable, and there are two in that group who may possibly be cured. The remainder had such extensive lesions that the likelihood of cure in these is very small.

In summary, then, it has been found that the photofluorographic method of examining the stomach results in a diagnostically satisfactory examination in 94 percent of the cases, and it is capable of detecting the presence of asymptomatic lesions in the statistically predictable number of instances.

Chairman Brunschwig: The next paper is by Dr. H. M. Pollard of Ann Arbor, Michigan, Cytologic Study of Gastric Aspirate for Carcinoma Cells.

CYTOLOGIC STUDY OF GASTRIC ASPIRATE FOR CARCINOMA CELLS [1] [2]

By Henry C. Bryant, M. D., William R. Craig, M. D., and H. Marvin Pollard, M. D., *Department of Pathology and Department of Internal Medicine, University Hospital, University of Michigan Medical School, Ann Arbor, Mich.*

Since July 1947, we have studied the cytology of gastric aspirated material in an attempt to evaluate this method of determining the presence of malignant cells in the fasting gastric contents. This study is the result of cytologic examinations to November 1948 and includes a comparison of the gastric residuum and the saline washings. We have correlated the final clinical diagnosis with the cytological findings, as well as the results of repeated aspirations in detecting malignant cells. The technique utilized in obtaining the fasting contents and staining the cells has been previously described (*1*, *2*).

This report now includes the material examined from 672 aspirations on 392 individual patients. We considered the residuum as that material aspirated after an overnight fast; whereas, the saline washings was that material obtained after forceful lavage several times with 100 cc. of saline and withdrawal of the remaining gastric contents. All patients had complaints referable to the gastrointestinal tract, or some condition was suspected in which a gastric analysis was felt to be of value in diagnosis. Due to our previous experience (*2*) an attempt has been made to wait in those cases with obstruction of the gastric outlet until the obstruction has been relieved or lessened because of the excessive foreign material otherwise present in the stomach.

In this study, the pathologist (H. C. B.) was not in possession of any clinical information at the time of examining the slides. His belief, however, is that the absence of this information has imposed an added hazard to accurate reporting rather than serving to abet scientific honesty. It is worthy of note that we have submitted to the pathologist material from various types of abnormal mucosal disease, the nature of which is shown in table 1.

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[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] The authors wish to express their appreciation for the excellent technical assistance offered by Audrey Green and Bernice Rochon.

TABLE 1.—*Cytologic examination of material obtained by gastric aspiration*

Diagnosis	Patients without gastric carcinoma clinically					
	Patients	Total aspirations	Result of cytologic study			
			Negative	Positive	Suspicious	Unsatisfactory
	Number	*Number*				
No disease	33	42	37	0	0	5
Gastric ulcer	54	108	67	3	3	35
Duodenal ulcer	73	101	73	4	13	11
Gastritis, hypertrophic	21	44	36	3	0	5
Gastritis, atrophic	14	40	15	8	5	12
Gastritis, superficial	1	6	5	0	0	1
Gastritis, catarrhal	2	5	1	4	0	0
Gastric polyp, benign	3	5	5	0	0	0
Polyposis, gastric	1	2	0	1	1	0
Pernicious anemia	4	6	3	0	0	0
Postoperative stomach (ulcer)	5	6	6	0	0	0
Gastric hemorrhage	6	7	5	0	2	0
Marginal ulcer	1	2	1	0	1	0
Oral sepsis	5	5	3	0	2	0
Hypertrophic pyloric stenosis	1	1	1	0	0	0
No gastrointestinal diagnosis	78	99	66	3	16	14
Nonspecific pneumonitis	3	3	2	0	0	1
Total	305	482	326	26	43	[1] 84
Percentage	------	(398)	81. 9	6. 5	10. 8	------

[1] Excluded from totals.

The results have been divided into the following categories for presentation:

(1) Those obtained in patients with no clinical evidence of cancer. It should be emphasized that this group includes patients with various forms of gastric disease such as atrophic and hypertrophic gastritis, and gastric and duodenal ulcer.

(2) Those from patients with clinical evidence of cancer of the stomach.

(3) Those from patients with histological evidence of gastric cancer.

(4) Comparison of the value of material obtained from the residuum and from saline washings.

(5) Results in repeated gastric aspirations in patients with histologic proven gastric cancer.

(6) Diagnoses in patients with false positive reports. The cytologic reports were correlated under numerous headings in an attempt to deter-

mine the efficacy of the procedure from the standpoint of percent positive, as well as percent false positive in cases with unproven gastric cancer.

There were 260 patients without a clinical diagnosis of gastric cancer by the usual methods of study on whom there was a negative or unsatisfactory cytologic study. Forty-six additional patients in the same category had at least one report which was either positive or suspicious for carcinoma cells in the gastric aspirate. These 305 patients have been consolidated and the results are shown in table 1.

Seventy-four patients were considered to have carcinoma of the stomach clinically, and 166 separate aspirations were performed on these 74 individuals. The results are shown in table 2. Sixty-eight were reported as negative in the cytologic study, 44 positive, 21 suspicious, and 33 were unsatisfactory. By excluding the unsatisfactory aspirations, a 33 percent correct diagnosis results when the total aspirations are used as the basis for the calculations.

The pathologist is of the opinion that a truer picture is presented when the histologic proof of gastric carcinoma is correlated with the cytologic study as shown in table 3. Forty-two patients had histologic evidence of gastric carcinoma, and in 5 of these the aspirated material was unsatisfactory for study. Thirteen of the 42 were negative, 6 were suspicious, and 18 were positive cytologically. Exclusion of the unsatisfactory aspirations results in a positive correlation by case of 48.6 percent.

Further analysis was made of the histologic evidence from the patients with tissue sections as follows: Metastatic carcinoma in the liver, lymph nodes, etc., compatible with origin in the stomach (14 cases); metastatic carcinoma, primary indeterminate (3 cases) with these 2 groups having X-ray or gastroscopic evidence of neoplasm of the stomach; and inconclusive (suspicious) for gastric carcinoma (2 cases). Inclusion of these patients in the group having primary gastric carcinoma results in agreement of a positive cytologic diagnosis with histologic proof of cancer in 45.5 percent

TABLE 2.—*Cytologic examination of material obtained by gastric aspiration*

Diagnosis	Patients with gastric carcinoma clinically					
	Patients	Total aspirations	Result of cytologic study			
			Negative	Positive	Suspicious	Unsatisfactory
	Number	*Number*				
Carcinoma of stomach	74	166	68	44	21	33
Percentage		(133)	51. 1	33. 0	15. 8	

TABLE 3.—*Correlation of results of cytologic study with diagnosis made by histologic examination*

Histologic diagnosis	Patients	Cytologic diagnosis				
		Positive		Suspicious	Negative	Unsatisfactory
	Number	*Number*	*Percent*			
Primary gastric carcinoma	42	18	48.6	6	13	5
Metastatic carcinoma compatible from stomach	14	5	------	0	9	0
Metastatic, primary indeterminate	3	0	------	2	0	1
Suspicious by tissue section	2	2	------	0	0	0
Total	61	25	45.5	8	22	[1] 6

[1] Excluded from the calculation.

Duplicate studies have been performed in 42 instances on patients with histologic proof of gastric carcinoma, and the results are shown in table 4.

TABLE 4.—*Comparison of results of cytologic studies of gastric aspirated material and of saline washings in patients with gastric carcinoma* [1]

Material studied	Results		
	Positive		Unsatisfactory
	Number	*Percent*	
Gastric aspirated residuum	9	28.1	10
Saline washing	9	26.4	8

[1] 42 duplicate studies.

Twenty-five patients with gastric carcinoma have had multiple aspirations in order to obtain more information on the efficacy of the procedure. As noted in table 5, new positives were still detected in the cytologic study in the fourth successive aspiration.

TABLE 5.—*Results of cytologic studies in successive gastric aspirations in patients with histologically-proved gastric carcinoma*

Aspiration	Patients aspirated	Second aspiration			Third aspiration		Fourth aspiration		Fifth aspiration	
		Positive	Positive new	Total	Positive new	Total	Positive new	Total	Positive new	Total
	Number									
First	61	19								
Second	25		2	7						
Third	12				3	4				
Fourth	7						1	2		
Fifth	4								0	1

Eighteen patients without gastric cancer by the present methods of clinical examination have had false positives on the initial report. This has been reduced to 12 with further study and reclassification in these patients reviewed without clinical material being given to the pathologist. This results in false positive report by aspirations of 5 percent and by case of 6.4 percent (table 6).

TABLE 6.—*Cytologic examination of material obtained by gastric aspiration*

Diagnosis	Patients with positive aspiration without gastric carcinoma clinically (306)		
	Patients	Aspirations	Positive
	Number	*Number*	*Number*
Gastric ulcer	2	4	3
Duodenal ulcer	4	14	4
Hypertrophic gastritis	3	11	3
Atrophic gastritis	3	23	8
Chronic catarrhal gastritis	2	5	4
Diffuse gastric polyposis	1	2	1
No gastrointestinal diagnosis	3	6	3
Total	18	65	26

DISCUSSION

It has been emphatically stated that this study was not undertaken as a survey procedure but to evaluate the accuracy of cytologic diagnosis. One would expect that with the presence of digestive activity of gastric juice and with the present methods of obtaining the exfoliated cells, the percentage of positives in known gastric carcinoma would be less than in the material obtained from the other anatomic regions (*2–5*). This was the basis for Zemansky's statement (*6*) in 1928 that the study of the gastric contents for cancer cells was of little value. He used the paraffin block method, however, in but a few cases. One of the inadequacies of the present method is exemplified by the poorer slides obtained from the residuum in which the exfoliated cells have been bathed in the gastric juice. Dr. W. C. Hunter reported in the discussion (*2*) that he and his coworker had more difficulty in establishing a positive cytologic diagnosis with the carcinomas with surface necrosis, and that has been our experience here.

The high percentage (20 percent) of false positive reports in certain cases of atrophic gastritis is of interest. This lesion, as considered by Schindler (*7*), may be the soil in which cancer can develop. We have used the term false positive to designate positive cytologic reports in patients with no clinical detectable evidence of cancer of the stomach at the present time. Dr. C. V. Weller [3] has pointed out, however, that these patients with false positive report at the present time may be found to have cancer at a later date. Histologic examination is not without similar difficulty. One case in particular was reported as showing changes which reminded the pathologist of carcinoma in situ of the breast, and he commented that further observation and experience were necessary to decide such issues. It is felt that all cases with false positive reports should be followed for 5 years.

Although duplicate studies would seem to show that there is no great advantage in one method of obtaining the exfoliated cells over the other, the pathologist believes that there is, in practice. The cell structure was better preserved, there was less digestion and less foreign material, and the slides were easier to interpret when the sediment was obtained from saline washings. In the previous communication (*2*) it was mentioned that no increase in efficiency was noted in the few stomachs lavaged with 75-percent alcohol, 5-percent sodium bicarbonate, or in secretions which resulted from histamine stimulation.

In certain selected cases cytologic examination should be a permanent part of our diagnostic armamentarium. As a survey procedure it is too time-consuming and as previously reported requires a pathologist well trained in the cytology of malignant cells to differentiate the border-line type (*9*). Hauptmann (*10*) has recently made a study of the cells obtained from surgical specimens by a special technique and obtained a high degree of correlation with histologic carcinomas.

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[3] Personal communication.

Six of the patients with false positive reports have had laparotomy for various reasons. In only one of them can the positive report be considered as the sole reason for the operation. This patient was a 59-year-old male with hepatomegaly, negative upper gastrointestinal radiographic examination, negative gastroscopic result, and with one positive and one negative cytologic report. At laparotomy he had a metastatic neoplasm of the liver. Careful exploration of the stomach, pancreas, gallbladder, and large bowel failed to reveal any primary site. The histologic report upon the biopsy specimen from the liver suggested neuroepithelioma or amelanotic melanoblastoma. No tissue was taken from the stomach at operation. Another patient had a penetrating posterior pyloric ulcer, a third patient had biliary cirrhosis (gastroscopic examination showed a suspected gastric carcinoma), and three patients had chronic catarrhal gastritis. One of the latter three had severe polypoid hyperplasia of the gastric mucosa, a second had cholelithiasis, and the third had an inactive duodenal ulcer with carcinoma suspected after gastroscopic examination. In six of these patients laparotomy was done during the early period of our cytologic study.

Eleven patients with a false positive report have been observed without laparotomy. Ten of these have been followed for 1 to 8 months by X-ray examinations, and so far the findings have been negative. Likewise, repeated cytologic examinations have resulted in a suspicious report in one case and negative results in five others. For the patient with the suspicious repeat examination, a similar report was made in the laboratory of Ruth Graham [4] after cytologic study of a gastric aspirate in November 1948.

Although it is inferred or stated that operations have been performed on the basis of positive cytologic reports alone, in cancer of the uterus (*3*), pre-invasive cancer of the stomach (*4*), and diverticulum of the bladder with carcinoma (*5*), we question whether a patient should be operated upon for suspected gastric cancer when the positive cytologic report is the only basis for the diagnosis.

SUMMARY

Cytologic examination of the fasting and lavaged gastric contents is capable of revealing gastric carcinoma cells.

The technique of conducting and examining the gastric contents is laborious, time-consuming, and demands the opinion of an experienced pathologist in the final analysis.

False positives may occur in a small percentage (5 percent) of patients in whom the presence of gastric cancer cannot be proved at the time.

Cytologic examination of the gastric contents has something to offer as an addition to our armamentarium in the diagnosis of gastric carcinoma. Its usefulness will probably be greater in those patients in whom gastric

[4] Jones, C. M.: Personal communication.

disease has already been suspected by other diagnostic measures. Particularly would this seem to be true at the present time in patients with atrophic gastritis.

In our study, carcinoma cells were found in 33 percent of the aspirations in patients with clinical evidence of gastric carcinoma, and in 48.6 percent of cases shown to have primary gastric carcinoma by histologic examination.

Examination of saline washings of the stomach added very little to the results of the examination of the fasting gastric residuum.

Repeated aspirations on separate days increased the number of positives which were detected in patients known clinically to have gastric carcinoma.

False positives were reported in patients with unproven gastric cancer from 6.5 percent of the gastric aspirations.

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Chairman Brunschwig: The next paper will be by Dr. Howard L. Richardson of Portland, Oregon; it is The Cytohistological Study of Gastric Washings.

THE CYTOHISTOLOGICAL STUDY OF GASTRIC WASHINGS[1][2]

By HOWARD L. RICHARDSON, M. D., *assistant professor of pathology, University of Oregon Medical School, Portland, Oregon*

A new approach to the study of gastric contents was carried out on 235 cases. The technique employed was to obtain the material for study by introducing 30 to 60 cc. of normal saline into a fasting stomach or following the multiple aspirations of an alcohol-histamine meal. The washings were immediately fixed in an equal volume of 10-percent formalin. This was followed by the addition of a saturated aqueous solution of picric acid which caused the mucus to solidify. The material was filtered, slowly dehydrated, and then handled as tissue. Sections (stained with a hematoxylin eosin and Orange G) were studied from three different portions of the paraffin block. The minimum distortion of the cell pattern and the study of a single layer enabled the following interpretations to be made regarding gastritis, ulcer, and carcinoma.

Aspirations from the normal stomach contain mucus, squamous cells, keratohyalin debris, and gastric mucosal cells (pl. 74, fig. 1). Fragments of mucosal epithelium are necessary for the diagnosis of acute or chronic gastritis.

In acute gastritis (pl. 74, fig. 2) interstitial edema is marked, and the epithelium contains intracytoplasmic granulocytes. In chronic gastritis the subepithelial stroma is infiltrated with plasma cells, lymphocytes, and granulocytes. The glands may demonstrate cyst dilatation and stromal inflammatory cells which collect in follicles (pl. 75, fig. 3). Atrophic gastritis presents many of the microscopic findings of chronic inflammation. However, there is a goblet cell change in the mucosal epithelium (pl. 76, fig. 4).

Ulceration within the gastric lumen is characterized by an organized fibrin mass in which granulocytes may be enmeshed (pl. 76, fig. 5). Intracytoplasmic hematin pigmentation may often be indicative of bleeding and, consequently, should be distinguished from anthrocotic pigment which is so common in the phagocytic granulocytes of sputum. The gastric epithelium directly surrounding the ulcer crater has been aspirated on several occasions, and this is found to demonstrate findings of rapidly regenerating tissue. This metaplastic epithelium was the cause of several of the false-positive cancer diagnoses.

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[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] This investigation was supported by a Cancer Teaching Grant, National Cancer Institute, U. S. Public Health Service, and aided by a grant of the Oregon Division, American Cancer Society, and by its Diagnostic Smear Laboratory, Mae S. Kuge, Technician.

Carcinoma is recognized by clumps of irregular large cells with hyperchromatic nuclei and prominent nucleoli (pl. 77, fig. 6, *A* and *B*). The influence of immediate fixation often makes these fragments superior to the surgical specimen.

This embedded section technique is not only widely applicable, but it enables the pathologist to examine a case in the minimum of time. The trauma of washing dislodges fragments of tissue from the gastric mucosa that are similar to biopsies and from these a cytohistological diagnosis can be made. After a 2-year study by this method, a sufficient amount of material was collected to discuss 78 cases, and the following figures are the result of that study.

When abnormalities are present in the stomach, they can be recognized in 90 percent of the cases. Gastritis was correctly diagnosed in 66.6 percent of the cases and ulcer in 83.3 percent. Carcinoma was diagnosed in 48.1 percent of the cases with a false-positive opinion in 3.9 percent.

A review of all slides proved that cell recognition improved with experience and that 66.7 percent of all cancer cases contained diagnostic masses of carcinoma cells in the embedded material. The inclusion of a cytohistological study would, therefore, seem to be an important adjunct in those cases in which the radiologist or gastroscopist discovers a lesion that is questionable.

Chairman Brunschwig: The next paper is by Dr. Cecil Patterson of Dallas, Texas.

PLATE 74

FIGURE 1.—A small fragment of gastric mucosal epithelium showing a normal arrangement of the columnar cells. ×120.

FIGURE 2.—A fragment of gastric mucosa which shows acute interstitial inflammatory reaction with transmission of granulocytes through the mucosal epithelium. ×120.

1

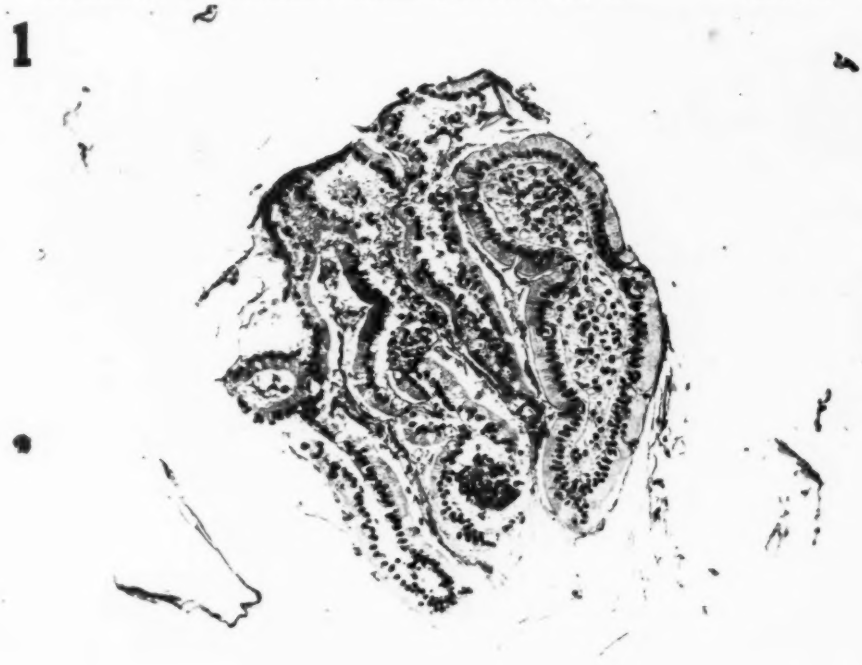

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PLATE 75

FIGURE 3.—A fragment of gastric mucosa which demonstrates dilated glands and stromal elements that contain an unusual number of lymphocytes and occasionally plasma cells. ×120.

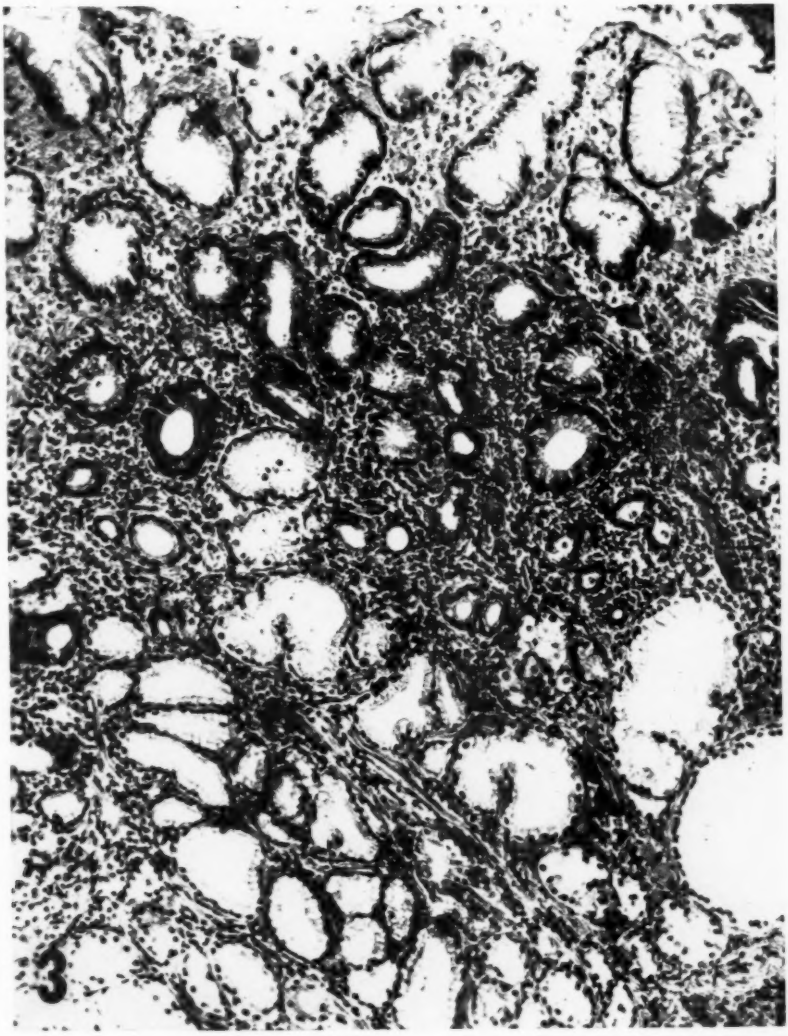
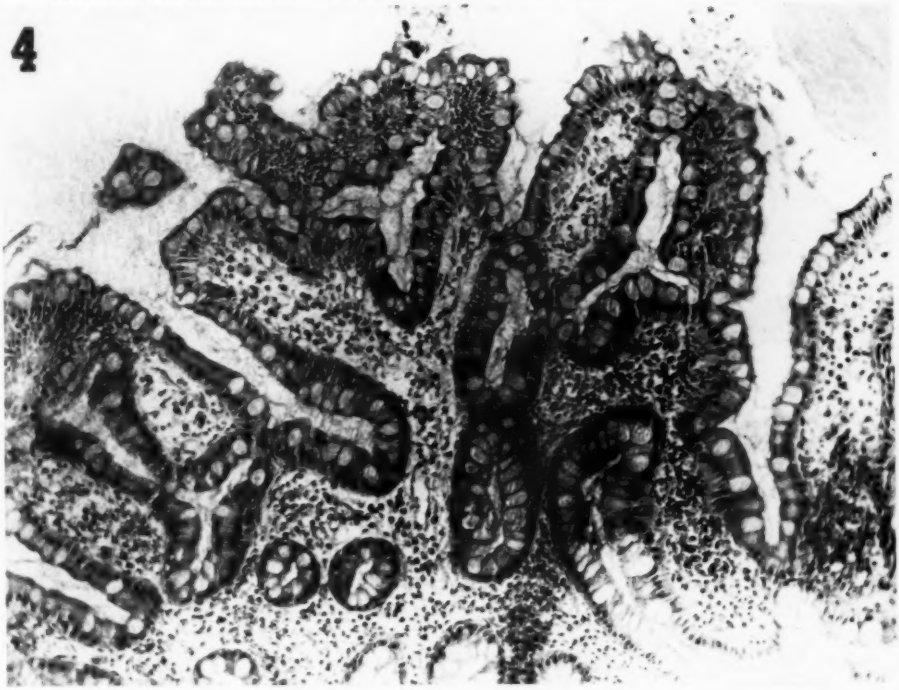


PLATE 76

FIGURE 4.—Goblet-cell metaplasia of the gastric epithelium indicative of atrophic gastritis. ×120.

FIGURE 5.—Ulceration may be manifested by an organized clump of nuclear debris within a fibrin network. ×120.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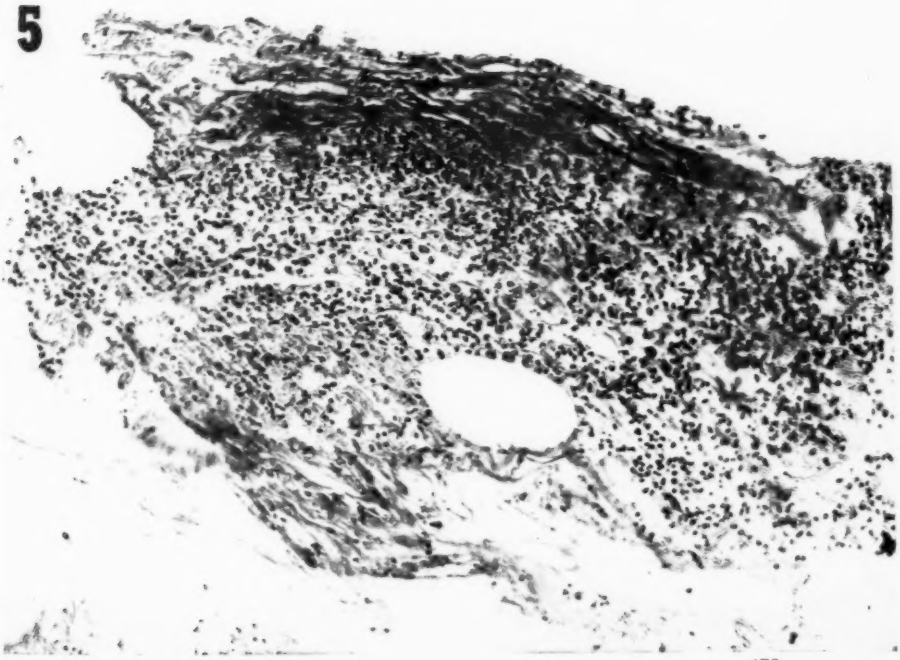
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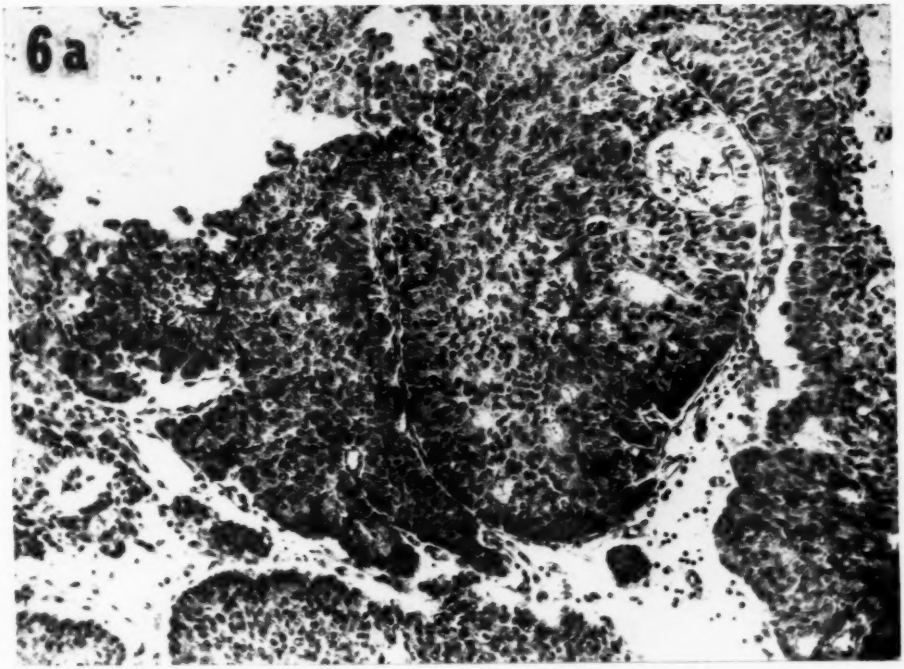
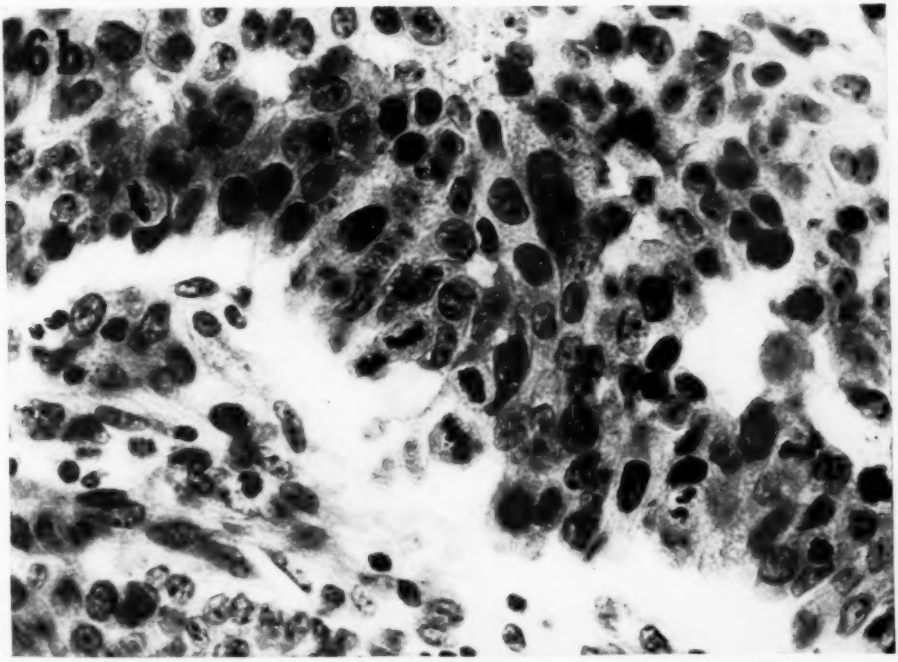
PLATE 77

FIGURE 6.—*A*, Carcinoma may be aspirated in amounts that are equivalent to a biopsy. ×120. *B*, A high-power microphotograph demonstrating mitotic figures within aspirated cancer-cell clumps. ×540.


6a


6b

USE OF THE ESOPHAGOSCOPE FOR OBTAINING BIOPSIES OF GASTRIC TUMORS [1]

By CECIL O. PATTERSON, M. D., F. A. C. P., *Department of Medicine, Southwestern Medical College, and the Gastroenterology Clinics at Parkland and Baylor University Hospitals, Dallas, Texas*

In most instances the open-end esophagoscope can be passed through the cardia. The esophago-gastric mucosal junction can be identified; also a small area near the cardia 5 or 6 cm. along the posterior wall and lesser curvature of the stomach can be visualized and is accessible for biopsy through the esophagoscope.

During 3 years (1946 to 1948, inclusive) positive biopsies were obtained through the esophagoscope as a part of the examination of 83 patients. Thirty-seven patients' lesions were in the upper two-thirds of the esophagus, and 46 patients' lesions were in the lower esophagus and fundic stomach. Of the 46 gastric and lower esophageal lesions, 22 proved to be neoplasms, 11 epidermoid carcinoma, and 11 adenocarcinoma. The remaining 24 biopsy diagnoses of the lower esophagus and stomach included 11 patients having peptic ulcer and 13 patients diagnosed variously as hyperplasia and hypertrophy of gastric mucosa and various inflammatory lesions as, for example, in hiatal hernia, and leukoplakia, keratosis, acanthosis, and scleradérma.

There were 2 unsatisfactory biopsy studies among the 83 patients. In 1, a 49-year-old white male, biopsies, repeated at an interval of 3 weeks from an ulcerating lesion high on the lesser curvature, showed only benign inflammatory tissue. Although the opinion of the surgeon at exploratory operation was that the lesion appeared benign, a nodule removed from the gastrohepatic omentum revealed metastatic adenocarcinoma. The second biopsy failure was from a suspicious lesion in the lower esophagus in a white male, age 43, reported as "adenocarcinoma, grade $\overline{\text{II}}$." When the surgeon opened and explored the esophagus and fundic stomach, no such lesion could be seen. The symptoms and suspicious appearing area were believed to be the result of inflammatory mucosa of hiatal hernia.

Of the 22 neoplasms in the region of the cardia, there were 4 instances in which no tumor tissue could be seen on first inspection, and the esophagoscope would not pass the obstruction. After gentle dilatations, positive biopsies were obtained in these patients. In 3 patients not included in the above 22, malignancy was presumed by inspection through the esophagoscope without biopsy. It was thought unwise to attempt biopsy through normal esophagus to get into a nodular underlying tumor at the

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

cardia. At operation the surgeons found malignant tumors in these 3 patients. The 11 biopsy diagnoses of adenocarcinoma at the cardiac end of the stomach were in patients who complained of dysphagia or had hematemesis and/or an X-ray deformity and were part of a group of 66 consecutive gastric carcinoma patients. Three of the 11 soon had successful resection of gastric fundic carcinoma. Four submitted to exploratory operations, and their gastric lesions were considered by surgeons to be inoperable. Four were advised by surgical consultants that the condition was inoperable because of a generally poor state, metastatic glands, or abdominal ascites of generalized carcinomatosis.

A 37-year-old white male, weight 187 pounds, was tentatively diagnosed as having cardiospasm. In the pictures, a small indentation was seen near the cardia on the lesser curvature of the stomach. Biopsy through the esophagoscope revealed adenocarocinoma, grade $\overline{\text{iii}}$. Operation was done elsewhere without delay, but metastases to regional nodes were reported. A 51-year-old white female had hematemesis a year previously and had dysphagia for 2 months. A small carcinoma high on the lesser curvature had been demonstrated by radiologic studies elsewhere. We verified the diagnosis of adenocarcinoma by biopsy through the esophagoscope, and the lesion was successfully resected by the surgeon.

A small leiomyoma diagnosed by X-ray and gastroscopy near the cardia did not come into view at esophagoscopy. The tumor was resected, verifying the diagnosis of gastric leiomyoma, a presarcoma lesion. Adenomatous polypi (*1*) of the stomach, which are notably precancerous, appeared in the middle third of the stomach and were not accessible to easy biopsy through the esophagoscope.

A not infrequent tumor-forming noncancerous lesion in the fundic stomach is that of nodular, hypertrophic, polypoid gastritis (*2*, *3*). Such multiple tumorlike lesions were studied by repeated biopsies in a 60-year-old white male who had lost 30 pounds and had recurring hematemesis. Later gastric resection verified the diagnosis of nodular and polypoid change of hypertrophic gastritis without evidence of neoplasm. We have observed a 22-year-old white female who had for 18 months recurring massive hematemesis and a large X-rayed deformity in the gastric fundus. At operation by the late Dr. Charles W. Flynn, the large bleeding "sows teat" (Ewald) soft tumorlike fundic masses were carefully biopsied and the stomach otherwise closed intact. Subsequently for 10 years on medical management there has been no recurrence of bleeding, and the patient has maintained normal weight of 120 pounds.

Because of the possible relationship to the genesis of gastric cancer, serial biopsies of the mucosa in gastric anacidity patients were studied during the progress of active gastric disease. For example, a 35-year-old white male complained of both ante- and post-cibum distress; he had lost 20 pounds and had anacidity to repeated histamine and insulin tests. A tender stomach and tender duodenal cap were noted at fluoroscopy. Biopsy was taken through the esophagoscope after gastroscopy had re-

vealed abnormal mucosa. The biopsy specimens revealed "active acute and sub-acute gastritis and normal appearing parietal cells" (*3–9*).

The esophagoscopic study of postoperative stenoses in the region of the cardia has been of value. In none of these was there a positive biopsy of recurring mucosal tumor. The course of the patients' conditions justified the assumption of obstructing tumor of probable metastases to regional nodes outside the lumen of the esophago-gastrostomy. Palliative dilatation of the strictures aided these patients in maintaining nutrition for many months; one patient continued productive work for 2 years.

In summary, the procedure of the biopsy of gastric tumors through the esophagoscope supplements the studies of gastric washings and other methods of diagnosis of gastric lesions (*10*). In experienced hands esophagoscopy provides a comparatively safe, quick, and concise method of clarifying diagnoses in the region of the gastric cardia, where unexplained dysphagia, or hematemesis, or obscure shadows to X-ray not infrequently present most crucial diagnostic problems. Esophagoscopy appears to improve our chances of getting a positive diagnosis of a small tumor earlier, when it is most likely to be curable by operation.

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Chairman Brunschwig: The next paper is by Dr. Charles Dunlap of New Orleans. It is Acid-Phosphatase Activity in the Gastric Content of Patients with Carcinoma of the Stomach.

ACID-PHOSPHATASE ACTIVITY IN THE GASTRIC CONTENT OF PATIENTS WITH CARCINOMA OF THE STOMACH[1,2,3]

By GEORGE W. CHANGUS[4] and CHARLES E. DUNLAP,
Department of Pathology, School of Medicine, Tulane University, Charity Hospital of Louisiana at New Orleans and U. S. Marine Hospital, New Orleans, La.

In 1941 Gomori (*1*) reported his method of staining for acid phosphatase in tissue sections. Among a variety of normal and malignant tissues which he studied, 9 of 11 carcinomas of the stomach contained large amounts of acid phosphatase not only in the neoplasm but also in the uninvolved gastric mucosa. Sections of normal stomach stained by the same technique showed little or no acid phosphatase. This remarkable finding led us to apply the acid-phosphatase stain to a number of specimens of carcinoma of the stomach, and by strict adherence to the details of the method, we were able in most instances to confirm Gomori's findings. This suggested that in patients with gastric carcinoma, enough acid phosphatase might escape into the lumen of the stomach to give the gastric contents an acid-phosphatase activity above the range of normal.

MATERIAL AND METHODS

Gastric contents were aspirated as completely as possible from 155 persons 12 to 16 hours after the last meal. Most of these persons were patients who had some gastric complaint. The gastric contents were placed in clean, dry bottles and the volume, color, and viscosity recorded. The specimens were centrifuged briefly to remove particulate matter, and the supernatant fluid was decanted and used for determinations of pH, free and total hydrochloric acid, occult blood, acid and alkaline phosphatase. The pH was determined with a Cambridge pH meter. The free and total hydrochloric acid was titrated with 0.01N sodium hydroxide using Topfer's reagent and phenolphthalein as indicators. Occult blood was detected by means of the benzidine reaction.

Acid and alkaline-phosphatase activities were determined by the methods of Gutman and Gutman (*2*) and King and Armstrong (*3*) respectively. The only modifications necessary were the substitution of 0.5 ml. of the supernatant fluid from centrifuged gastric aspirates for the 0.5 ml. of blood serum used in the original procedures and the arbi-

[1] Presented at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] This work was supported in part by a research grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and in part by the Margaret W. Keller Gift for Cancer Research.

[3] The authors wish to express their gratitude to Dr. Stanley L. Rea for his help during the early stages of this study, to the many clinical colleagues who contributed specimens, and to Dorothy Ann Lesniak and Winifred Seegers for faithful and dependable assistance in the chemical and technical work.

[4] Fellow of the American Cancer Society.

trary use of an incubation period of exactly 1 hour. A control tube containing the buffer-substrate was incubated at the same time, and at the end of one hour 0.5 ml. of the supernatant gastric fluid was added. In both the acid and alkaline phosphatase determinations the units were defined as the number of milligrams of phenol liberated from the disodium phenyl phosphate substrate per hundred milliliters of gastric fluid during 1 hour incubation at 37° C., at pH 4.9 and 9.0 respectively. Duplicate determinations of the acid-phosphatase activity were done on 28 samples in order to test the reproducibility of the results.

Samples of gastric aspirates of high acid-phosphatase activity were stored in the refrigerator at 4° C., held at room temperature (21° and 26°), or incubated at 37° for varying periods to determine the loss of enzymatic activity. Aliquots of other samples were adjusted to pH values ranging from 1.5 to 8.0 by the addition of 0.1N hydrochloric acid or 0.1N sodium hydroxide and then tested for acid-phosphatase activity in order to determine the stability of the enzyme within the pH range encountered in the gastric contents of our patients. Pilot tests were made according to the technique of Bensley, Wood, and Lang (*4*) of the effect of added 0.5-percent formaldehyde on gastric, biliary, and red-cell acid phosphatases.

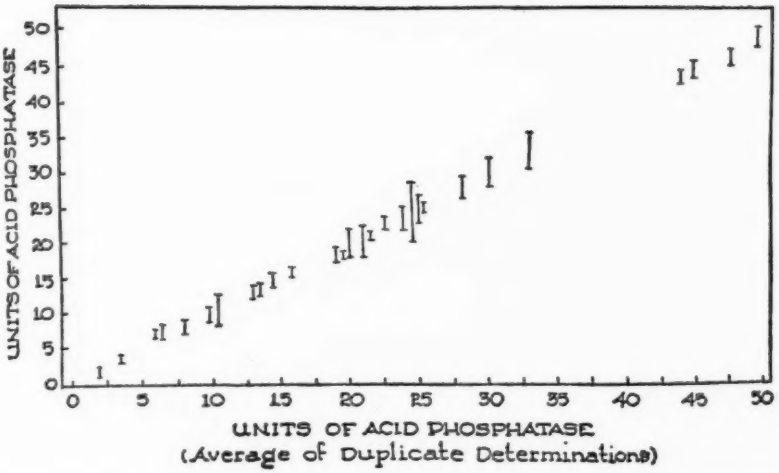
RESULTS

Alkaline-phosphatase activities determined on portions of the same samples used for acid phosphatase studies gave values which were higher, on the average, in patients with gastric carcinoma. However, there was considerable overlapping of the values obtained in the cancer and non-cancer patients.

Duplicate determinations on 28 samples of gastric contents gave values with an average difference of 2.7 units of acid phosphatase (text-fig. 1). Since large discrepancies were not encountered, it appears that this source of experimental error is not of great consequence.

The loss of acid-phosphatase activity on storage was not identical for all specimens held for the same time at the same temperature. Identical changes would scarcely be expected in crude gastric contents which differed in pH, organic and inorganic constituents and in the degree and nature of bacterial contamination. However, the loss in activity was roughly 7 percent on 24-hour storage at 4° C., 70 percent on 24-hour storage at 21° and 26°, and 50 percent on 1 hour incubation at 37°.

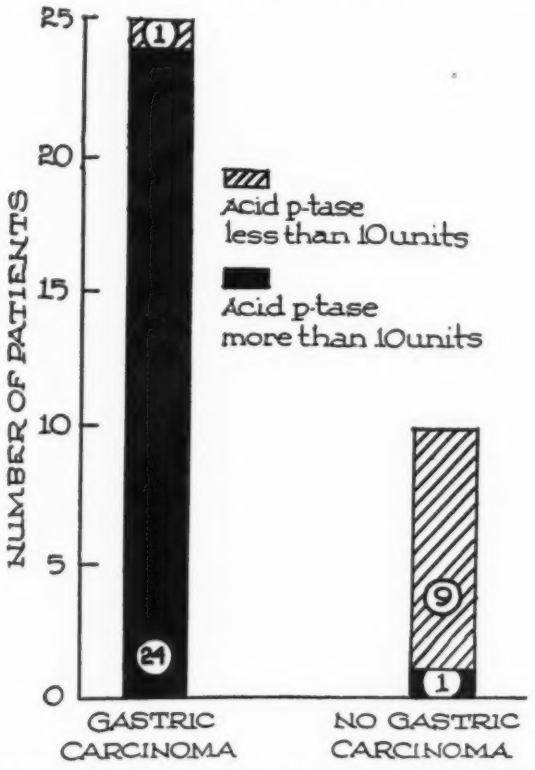
Since red blood cells and bile contain acid phosphatase (*5*), their presence in aspirated gastric contents might well introduce a significant error. A method of eliminating possible errors due to acid phosphatase of extragastric origin is suggested by recent reports of the selective inactivation of red-cell acid phosphatase and partial inactivation of bile acid phosphatase by 0.5-percent formaldehyde (*4*, *5*). Preliminary studies indicate that gastric acid phosphatase, like that originating from prostatic carcinoma, is relatively stable during brief exposure to this concentration of formaldehyde. In the small number of samples from patients with



TEXT-FIGURE 1.—Variations in acid phosphatase value found on duplicate determinations on 28 samples of gastric contents. In each case the difference in values found in duplicate determinations is represented by the length of the bar. The cases are arranged along the abscissa in order of increasing acid-phosphatase activity. The average difference between duplicate determinations was 2.7 units.

gastric cancer in which formaldehyde inactivation has been done according to the method of Bensley, Wood, and Lange (4) residual acid-phosphatase activity has remained high.

Aliquots of gastric contents from eight different patients were adjusted to pH values ranging from 1.5 to 8.0 by the addition of 0.1N hydrochloric acid or 0.1N sodium hydroxide. These samples had initial acid-phosphatase activity ranging from 10 to 46 units and original pH values of 4.6 to 8.0. After the pH was adjusted to the desired levels in each case, the aliquots were allowed to stand at room temperature for 30 minutes, and the pH and acid-phosphatase activity were then determined again. It was found that a stable level of enzyme activity was maintained between pH 3.5 and 8.0 except for a suggestion of a slight rise near pH 4.0. Below pH 3.5 there was rapid inactivation which was complete in solutions more acid than pH 2.0 (text-fig. 2). The inactivation may represent either a direct effect of acidity or an indirect effect of other substances present in crude gastric contents which act on acid phosphatase at the lowered pH. The observations on acid inactivation in vitro are well supported by the results of routine determinations on fresh specimens of gastric contents. Significant amounts of acid phosphatase were never demonstrated in samples with an initial pH of less than 2.0. The results were variable on samples with a pH between 2.5 and 3.5, and in samples more alkaline than pH 3.5 the pH had no apparent relationship to the level of acid-phosphatase activity. In general those samples that showed any free acid had a pH less than 3.5 and were hence unsuitable for acid-phosphatase determinations.



TEXT-FIGURE 2.—Percent in vitro inactivation of acid phosphatase in gastric contents with change in pH. Eight samples of gastric contents with initial acid phosphatase values ranging from 10 to 46 units and initial pH of 4.6 to 8.0 were used. The pH was adjusted to the required values by addition of 0.1N HCl or 0.1N NaOH. The acid phosphatase was again determined after the specimen had stood at room temperature for 30 minutes. The results are expressed as the percent of the original activity remaining.

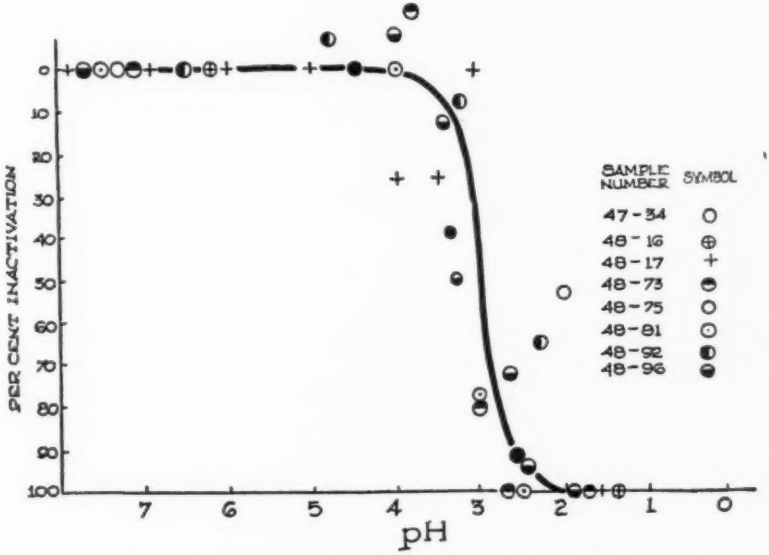
Another source of error was not found until a great many determinations had been done. In reviewing those results that departed widely from the general trend, it was noted that the original volume of fluid aspirated from the stomach was in many instances only a few milliliters. Since complete aspiration was attempted in all instances, such small samples might easily represent the product of local regions of the gastric mucosa and not a representative and well-mixed sample of the gastric content. All results were, therefore, excluded that were derived from original aspirates of less than 10 ml. in total volume.

In evaluating the relationship between gastric carcinoma and the acid-phosphatase activity of the gastric contents, it was decided to eliminate 120 of the 155 determinations as uninformative or undependable for one or more of the following reasons:

(1) Sample of gastric contents unsatisfactory—(a) initial pH less than 3.5; (b) volume less than 10 ml.; (c) held at room temperature over 2 hours; (d) held in refrigerator over 48 hours; (e) gross contamination with blood or bile.

(2) No opportunity for pathologic examination of the stomach.

Of the 35 samples of gastric contents which were considered satisfactory, 25 were obtained from patients with pathologically confirmed carcinoma of the stomach and 10 from patients without gastric carcinoma. The latter group was made up of 3 patients with benign gastric ulcers, 2 with chronic gastritis, 1 with duodenal ulcer, 1 with a large pancreatic rest of the antrum of the stomach, 1 with squamous-cell carcinoma of the lower end of the esophagus, and 2 with pernicious anemia. The 2 patients with pernicious anemia are the only ones in either group that lack pathological confirmation and were included because of interest in the relationship of pernicious anemia to gastric cancer (*6*). In 9 of the 10 patients without carcinoma of the stomach the acid-phosphatase activity of the gastric contents was from 0 to 9 units per hundred milliliters. One patient with a squamous-cell carcinoma of the lower end of the esophagus had 15 units. In all but 1 of the patients with gastric carcinoma the acid-phosphatase activity fell between 10 and 46 units. From these data an arbitrary value of 10 units of acid phosphatase was selected as the dividing line between those patients likely to have cancer and those whose gastric complaints were probably due to some other disorder (text-fig. 3). In 9 cases the



TEXT-FIGURE 3.—Acid-phosphatase activity of gastric contents of 35 patients, 25 with pathologically confirmed carcinoma of the stomach and 10 without gastric malignancy.

acid-phosphatase values were so close to 10 (9–12) that the experimental error might have thrown them on either side of this arbitrary dividing line.

This report is based on only a small group of patients including no early carcinomas of the stomach. Only 10 patients without carcinoma could be included since pathologic studies are lacking in the others. In our series the gastric contents of most achlorhydric patients with gastric carcinoma gave acid-phosphatase values above 10 units. One patient without gastric carcinoma also had a high value. A number of factors which might affect the results have not been adequately controlled; for example, digestive enzymes, intragastric dilution, regurgitated duodenal contents, swallowed mucus and saliva, and other substances which may be present in the gastric contents. The importance of these and other factors must be known and a much larger series of patients studied before any reliance can be placed on acid-phosphatase determinations as an indication of the presence or absence of gastric carcinoma.

SUMMARY

The acid-phosphatase levels in the gastric contents of 155 patients with and without gastric carcinoma were determined. All the gastric samples were aspirated 12 to 16 hours after the last meal. Samples with a low pH were found to have little or no acid-phosphatase activity. When samples with a high original acid-phosphatase activity were adjusted to lower pH levels, it was found that the activity fell off sharply as the pH fell below 3.5. At pH below 2.0 there was almost 100 percent inactivation of acid phosphatase. Gastric contents left standing at room temperature for several hours or left in the refrigerator over 48 hours showed a substantial loss in acid-phosphatase activity. Gastric aspirates of less than 10 ml. total volume often contained much mucus and gave undependable results. It was concluded that they did not represent well mixed samples of the gastric content. Samples containing gross blood and bile were of doubtful value since bile and red blood cells contain acid phosphatase. It was, therefore, decided to exclude as unsatisfactory all samples which contained free acid (pH less than 3.5), had been standing at room temperature over 2 hours or in the refrigerator over 48 hours, had a volume of less than 10 ml., or contained gross blood or bile. Other samples were excluded because no pathologic confirmation of the clinical diagnosis was available.

The gastric aspirates of 35 patients were considered acceptable for analysis. Among the 25 patients with pathologically confirmed gastric carcinoma the acid-phosphatase level in the gastric content was over 10 units per 100 ml. in 24 and below 10 units in 1. Ten patients had no gastric carcinoma, and of these the acid-phosphatase activity was less than 10 units in 9 and above 10 units in 1.

Enzyme determinations on fluids of such complex and variable composition as gastric contents should be interpreted with caution. No cases of early cancer have been studied and many variables have not been controlled. The data at hand, while promising, do not warrant the use of acid-phosphatase determinations as a clinical test for carcinoma of the stomach.

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Chairman Brunschwig: There is only one paper left in the series; perhaps we had better hear that before we open the discussion. The next paper is by Dr. Freddy Homburger of Boston, Mass., Further Studies on Metabolic Changes in Patients with Gastric Cancer.

FURTHER STUDIES ON METABOLIC CHANGES IN PATIENTS WITH GASTRIC CANCER [1] [2]

By F. Homburger, M. D.,[3] Nelson F. Young, Ph. D.,[4] Aurelia Potor, M. D., Jack B. Trunnell, M. D., Benedict J. Duffy, Jr., M. D., Mary L. Petermann, Ph. D., and C. P. Rhoads, M. D., *Department of Clinical Investigation of the Sloan-Kettering Institute for Cancer Research, New York*

Studies on metabolic changes in patients with cancer carried out at the Memorial Hospital for nearly a decade have been continued with the dual aim of improving the pre- and postoperative management of patients with gastric cancer and of acquiring knowledge on some basic aspects of the disease. This paper represents a brief progress report covering the period since April 1947, when a publication summarizing previous work of this group was published in this Journal (*1*).

CHANGES IN CARBOHYDRATE METABOLISM

The clinical studies which had shown defective hepatic glycogenesis in patients with gastric cancer corrigible by injection of adrenal cortical extract (*2*) were extended to experimental animals. The liver of mice bearing sarcoma 180 (*3*) exhibited impairment of hepatic glycogenesis responding to adrenal cortical extract comparable to the results previously obtained in humans. The administration of adrenocorticotropic pituitary hormone (ACTH) to mice bearing sarcoma 180 did not improve hepatic glycogenesis unless the hormone was given prior to tumor implantation.[5] Some pilot experiments were made using ACTH in patients with cancer to study hepatic glycogenesis.

CHANGES IN PROTEIN METABOLISM IN PATIENTS WITH GASTRIC CANCER

Nitrogen and mineral-balance studies in patients with gastric cancer were extended to last as long as 350 days in one case and 127 days in another. Electrophoretic studies of the plasma were done periodically, together with measurements of plasma volume. In spite of positive

[1] Read by Dr. Homburger at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 13, 1948.

[2] These studies were aided by grants from The National Cancer Institute, National Institutes of Health, Public Health Service; The Teagle Foundation, Inc.; The New York City Cancer Committee of the American Cancer Society, Inc.; James Foundation of New York, Inc.; U. S. Navy, Office of Naval Research; American Contract Bridge League; American Cancer Society, Inc.; and Winfield Foundation.

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[4] Present address: Medical College of the University of Virginia, Richmond, Va.

[5] Homburger, F., and Young, N. F.: Unpublished data.

nitrogen balance, no regeneration of plasma protein was found, and the plasma albumin level remained low.

Defective synthesis or altered distribution of plasma proteins were postulated. Experiments were done on mice bearing sarcoma 180 to study the distribution between organs of nitrogen in these experimental animals. It was found that in mice bearing sarcoma 180 or a transplantable adenocarcinoma of the breast, there occurred marked hyperplasia of the lymphoid tissue (*4*). Simultaneously there was a marked increase in size and in nitrogen concentrations in the spleen and liver of tumor-bearing mice as compared with pair-fed controls. No hypoproteinemia was found in these animals.[6]

In 12 patients who had survived partial or subtotal gastrectomy for cancer for from 5 to 15 years, electrophoresis of blood plasma, hematological studies, and studies of liver functions revealed no abnormalities. There was, however, complete achlorhydria after histamine in all these patients. This eliminates achlorhydria as an etiological or pathogenic factor in the disturbances of protein metabolism of patients with gastric cancer.[6]

ALTERATIONS OF ELECTROLYTE EXCHANGES

Quantitative sodium chloride and water balance studies were performed on two subjects with gastric cancer, one patient with Addison's disease, and a young healthy individual. They received daily increasing doses of salt, the fluid intake being maintained constant at 3,000 ml. per day throughout the experiment. Combined urinary and fecal output was measured and compared with the intake. In the normal subject, the slightly positive sodium balance could probably be accounted for by the NaCl lost in the sweat, which was not measured. In the subject with Addison's disease, there was an excess excretion of about 200 meq. of sodium in the first 2 days of the experiment, while the sodium intake was 150 and 300 meq. Later, as the intake increased up to 1,100 meq. per day, a marked retention of sodium occurred. In both patients with gastric cancer, the sodium output lagged behind the intake over the period of study.

The differences in the rates of intake and output obtained in the various patients are statistically significant. Whether they are so biologically remains to be seen. It would seem from this experiment that there is a decreased tolerance for salt, i. e., increased retention in patients with gastric cancer, and that they occupy in this respect an intermediate position between the patient with adrenal insufficiency and the normal subject. This was borne out by biopsy studies done at the beginning and at the end of the experiment and analyzed for moisture, sodium chloride, and potassium.

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[6] HOMBURGER, F.: Unpublished data.

SUMMARY

Continued studies on metabolic changes in patients with gastric cancer have further defined the systemic syndrome found in these patients.

Some of these changes (hypoproteinemia, anemia, and liver dysfunction) were no longer found 5–15 years postoperatively in subjects cured of the disease. The finding of the patients' inability to replenish plasma albumin even a year postoperatively and the existence of a tendency to salt retention should be kept in mind in the postoperative management of patients with gastric cancer.

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Chairman Brunschwig: We will go right ahead with the discussion of all the papers given this afternoon. I will take the liberty of calling on some of you first. Dr. Pack.

Dr. Pack: About 15 years ago Dr. Harry Hauser, now in Cleveland, was a Memorial fellow in radiology. On his own initiative he ran a series of 100 patients more than 40 years of age who had no symptoms suggesting gastric disease. These patients were studied by fluoroscopy and serial roentgenograms of the stomach. In that group of slightly less than 100 patients he did find 1 patient who had a small polypoid tumor. Gastric analysis was subsequently done and showed that this patient had achlorhydria. I removed that small tumor of the stomach and it was carcinoma. That patient is living now more than 14 years later. That was the first experiment I know of the so-called group survey of well patients, but it was not followed up at our institution.

The classical experiment of Drs. St. John, Swenson, and others, of course, is familiar to all of us. As a practical method for detecting early cancer of the stomach, I think we can quote a later statement by Dr. Swenson, himself, who called attention to the fact that there are in the United States 42 million Americans living who are more than 40 years of age; and, assuming that this method of attempting the early detection of unrecognized gastric cancer would be universally applied, and allowing 5 minutes for a rapid fluoroscopic survey of a single patient, and permitting the roentgenologist to work union labor hours of 40 hours a week, it would require the full-time services of some 1,800 roentgenologists to survey this great group every year.

Apropos of that, he said that although it required 1,800 roentgenologists to make the survey, there were only 2,300 who were diplomates of the Board of Radiology; so as a practical method we cannot look for its universal application to improve perceptibly the percentage of early diagnoses, and, of course, with that an opportunity to increase the percentage of cures.

Perhaps this is one fault of the various cancer detection clinics which are now so popular throughout the country. If one requests the various heads of these clinics to

estimate the total cost, of finding an undetected cancer, we find that in many instances it amounts to as much as $10,000.

Last month, when I was in Providence, R. I., I was discussing this problem with some of the doctors who have been engaged in this work at Pawtucket, one of the earliest tumor clinics in general hospitals that has been established in this country. At Pawtucket, for example, in the cancer detection clinic, three doctors, all well trained in cancer diagnosis, working three afternoons a week for the entire year, reported by the end of the year not one major cancer had been diagnosed of those patients who came to the cancer detection clinic, presenting no signs or symptoms suggesting the presence of a cancer or that made the patients suspicious of cancer.

In talking it over at that time, it was suggested, as has been suggested elsewhere, that it might be a better practical policy to offer to the physicians throughout the State of Rhode Island or any other state for that matter, a flat sum of $25 for the first doctor making a report of a proven case of cancer to the state committee. That, in the State of Rhode Island, where there are 1,100 reported cancers a year, would cost the state cancer committee about $27,500, or what it would roughly cost to find 3 unsuspected gastric cancers.

I mention this as a practical means for diagnosis because, in the State of Rhode Island all cancers are required to be reported to the State, as I understand they are in Connecticut, and as they are in the State of New York exclusive of New York City.

I do not mean to say that the cancer detection clinic is not worth while. There are many other beneficial activities for the diagnosis of other diseases; but, practically, or realistically, something should be done about it, and the way has been pointed here today by two very important papers, one from the University of Minnesota, in which the screening process has been applied and studies have been done only on those who have achlorhydria.

Obviously, the incidence of cancer here is relatively high, as I understand, 8 to 10 times as great as the general population.

I know the figures we have at Memorial Hospital are slightly higher than those of Dr. Wangensteen at the University of Minnesota. I understand that about 8 percent of our patients with gastric cancer have hyperacidity, and 48 percent of our cases have acidity, using the histamine test.

The second method of screening, which I think is a matter of great economic importance and I stress this economic importance because, after all, we realize that there are so many patients with cancer in this country who need treatment, so many with very advanced cancers who need care and hospitalization, is that it requires money and the services of doctors. Although the cost of $10,000 to discover early cancer of the stomach, from the point of view of the patient who has the cancer, is a very good investment, whether it comes from his pocket or the pocket of the state or the pocket of the Cancer Society, still, in considering economics of the problem as a whole the country over, something should be done to lessen the cost of this discovery. This method of making the small roentgenogram of the stomach as a means of screening out the great number of patients who have normal stomachs is an important factor in economy.

We are all interested in the exfoliative cytology of the stomach as a means of diagnosis. I remember years ago in the laboratory of Memorial Hospital when Dr. James Ewing was active and his laboratory technician, Mr. Edward Ellis, who worked with him more than 30 years, called to my attention on several occasions specimens of gastric juice sent up for routine analysis, stating, "Dr. Pack, I am sure that patient has cancer of the stomach because the study of cells in the stomach shows definite cancer cells." These remarks were being made by a laboratory technician of unusual experience due to his long association with Dr. Ewing—and I wasn't sufficiently alert at that time to pay much attention to the discovery, except that it was of considerable interest, not realizing its practical importance.

After the great interest in the Papanicolaou method became evident, I went back to the old laboratory sheets of those days and found that 20 years ago, in 1928, microscopic slides prepared of the sediment that showed these cancer cells very well with the diagnoses made of gastric cancer confirmed by Dr. Ewing. It is with considerable chagrin that I report I was not sufficiently curious or alerted to the possibilities of using this more or less as a routine method. Dr. Homburger's work at Memorial Hospital represented collaboration between the Laboratory of Clinical Investigation and the Gastric Service of Memorial Hospital. It has been of great practical importance in the care of our patients, both preoperatively and postoperatively. I believe that these studies on the metabolic changes incident to the presence of gastric cancer have enabled us to treat the patients more intelligently to lessen the morbidity and mortality of those patients in the surgical treatment of this disease.

Chairman Brunschwig: Is there anyone else?

Dr. Wangensteen: Dr. Gomori is here.

Chairman Brunschwig: Dr. Gomori, would you like to comment?

Dr. Gomori: I would like to make a remark in respect to the paper of Dr. Dunlap. About 5 years ago I did about 120 determinations of acid-phosphatase activity on gastric juices, and I am sure of the diagnoses in at least 20 or 25 of the cases. My results were very different from those obtained by Dr. Dunlap except for one point on which the results were identical. I realized very soon that gastric juice, on account of its acidity, is unsuitable for this technique. In not one of the cases did I have an activity of over 10 units per 100 c.c., and I did not find any significant difference between cancer and noncancer, so I dropped the whole project after quite a large number of determinations. In trying to explain the difference, I can't find any other important point but the age of the specimens. I did not pay any attention to the 2-hour interval Dr. Dunlap considers important and my specimens probably were staler than that, maybe 3 or 4 hours old. I usually received specimens obtained in the morning in the early afternoon hours.

Dr. Ivy: I should like to direct a question to Dr. State. I am not clear on the relative percentages of the incidence of cancer of the stomach, in patients with and without dyspeptic symptoms, and in those with achlorhydria, and in those with pernicious anemia. I should like to know whether or not the relevant percentages indicate that the incidence of cancer of the stomach is higher in patients with pernicious anemia than in patients with achlorhydria. For instance, in the St. Johns' group, the incidence of cancer was approximately one-tenth percent. In your achlorhydrias it was approximately 1 percent. In 92 patients with pernicious anemia there were 3 cases with cancer; that is about 3 percent. I realize that those percentages are quite small. The differences may not be significant statistically, but my question is: Does the presence of pernicious anemia add increased gastric cancer liability to the presence of achlorhydria alone?

Chairman Brunschwig: Dr. State, do you want to answer that now?

Dr. State: I think that might be answered while Dr. Rigler is here. It was his study that introduced the problem.

Chairman Brunschwig: Dr. Rigler.

Dr. Rigler: Our present figures for the incidence of carcinoma of the stomach in patients with pernicious anemia are about 6 percent. Dr. State is reporting only a small segment of the pernicious anemia cases because his group started studying them at a certain period of time. Figures on the patients with achlorhydria or low free hydrochloric acid were close to 1 percent. I do not know what the incidence would be in any average group of clinic patients. Do you, Dr. State?

Dr. State: No, I don't have them.

Dr. Rigler: I don't know what that is. In our cases I don't know what that would mean.

Dr. Ivy: I had in mind the figures of St. John, where in patients free of dyspeptic symptoms the incidence of gastric cancer was about one-tenth percent. In your group

of achlorhydric patients the incidence was approximately 1 percent; in the pernicious anemia patient, the incidence is 3 or 6 percent.

Dr. Rigler: I was going to discuss that. There is a difference in the groups of patients with regard to the degree of achlorhydria or low free hydrochloric acid. We increase the number of carcinomas which would be included in the group to a considerable degree; probably about 90 percent of all the carcinomas are included within the group if the high limit is set at 30 units, while the number of cases which need to be examined is only increased from about 25 to 32 percent of the population.

In explaining the difference between our figures and those of others it should be pointed out that we have made a more extensive, more elaborate examination than has been made on any of the other surveys. That is, we have done fluoroscopy, spot films, and routine roentgenograms. The possibilities are, therefore, that we might discover one or two more cases than would have been discovered in the course of routine simple fluoroscopic examination. That is a personal opinion, but I feel confident that it may represent a part of the discrepancy.

There is another possibility, and that is that in Minnesota we are dealing with a Scandinavian population where the incidence of carcinomas might be somewhat greater than in other population groups.

Chairman Brunschwig: Dr. Golden. (Dr. Ross Golden, New York.)

Dr. Golden: I would like to ask Dr. Homburger whether the systemic disturbances he described disappeared or diminished when the gastric resection was done and, if so, whether they recurred as metastasis developed. In regard to the studies of the cells in the gastric juice, it would be of additional interest if, in the reported cases, the size of the growth could be correlated with the frequency of the appearance of cancer cells.

The majority of the extensive carcinomas can be detected relatively easily by X-ray methods. We need the most help, from the practical standpoint, in the detection of those of small extent. It would be helpful in the correlation of X-ray findings with the carcinoma demonstrated on the specimen if the area of the inner surface involved with the growth could be clearly delineated. It is difficult, because of the contraction of the muscle of the stomach wall, to make an exact correlation with the shadows of the living stomachs.

Chairman Brunschwig: Dr. Homburger, do you want to answer that?

Dr. Homburger: The first part of your question is very difficult to answer. We only know that in a period of about a year following gastrectomy, some of these changes did persist, and we know, on the other hand, that in a group of people who have survived from 10 to 15 years, none of the changes such as we could measure preoperatively in other patients were found after this length of time. Of course, we did not know in these same cases whether they were present before the operation.

Chairman Brunschwig: Dr. Schiff.

Dr. Schiff: We followed a group of 15 patients with achlorhydria for 3 to 8 years with repeated gastroscopic examination, X-ray studies, and repeated gastric analyses, and at the end of the 8-year period none of these patients had developed either a recognizable gastric polyp or a carcinoma. We heard from one patient, whom we last saw 6 years ago, just within the past 2 months, and that patient had developed a gastric carcinoma. In the last 2 years I have seen 5 patients develop gastric carcinoma who had had achlorhydria anywhere from 3 to 12 years before. On previous X-ray and gastroscopic examinations there was no evidence of tumor.

I would like to ask Dr. Pollard if in any of his patients with a positive Papanicolaou smear the diagnosis could not have been made by other laboratory means such as the X-ray, for example.

With reference to Dr. Patterson's remarks, our surgical service in collaboration with the pathologic service has recently studied six cases of gastric cancer in the proximal half of the stomach. In five of the six cases there was microscopic extension into the lower end of the esophagus well beyond the gross boundary of the tumor. I wonder

if it would be feasible, in such cases, to consider biopsy of the lower end of the esophagus in order to detect such microscopic extension of the tumor in order to help guide the surgeon in the degree of resection he is to carry out.

Chairman Brunschwig: Dr. Pollard.

Dr. Pollard: In answer to Dr. Golden's statement, we have actually not correlated our results with the actual size of the gastric tumor, and conceivably this might add some valuable information. For example, it would be highly important to know that an extremely small tumor could be capable of exfoliating cells which could be detected in the aspirated material.

We also have not done a survey on so-called asymptomatic individuals. Such a survey would add valuable information, and we contemplate undertaking this in the near future. Our primary purpose in the present study was to determine what our percentage of positives would be in clinically proven cases with gastric carcinoma and at the same time to ascertain the number of false positives.

I believe that this also answers Dr. Schiff's question in that we have avoided a study of individuals without known gastric disease. I would like to re-emphasize the point that our pathologists have been confronted with various types of gastric pathology in making our cytologic study.

Chairman Brunschwig: Dr. Richardson.

Dr. Richardson: I might say to Dr. Golden's question that we have made a correlated study between the size of the lesion and the material aspirated. We have found that in the material aspirated from the large fungating necrotic carcinomas of the stomach you will seldom be able to detect cancer cells. All you will detect is necrotic protein, ghost cellular masses, and debris. In the carcinomas of the stomach (from which I must exclude the scirrhous type or the one that infiltrates), we have made one early diagnosis. This case, which was the first slide I showed you, was a small papillary mass, and our first aspiration showed this large group of carcinoma cells. On X-ray, however, no lesion was demonstrated. X-ray, as you already know, is considered the standard for accurate gastric visualization, so it was felt that we were wrong. We proceeded, nonetheless, to do seven other aspirations on this case, and in each aspiration we found quantities of granulocytes, fibrin, and protein. We knew we were dealing with a stomach that contained a lesion.

The surgeon finally opened the stomach and in the prepyloric area found a papillary adenocarcinoma no larger than 1 cm. The patient recovered, but 8 months later died. On autopsy it was found that death was due to coronary thrombosis. There was no evidence of metastasis.

Dr. Rigler: I failed to mention one other point in answer to Dr. Ivy's question; that is, in trying to explain the difference between our figures and those previously reported on surveys. Our figures represent the number of patients but not the number of examinations. Many of these have been repeatedly examined; in some cases of pernicious anemia 12 examinations have already been done. In the figures presented by Drs. St. John, Swenson, and Harvey, only one examination was made. Some of our patients developed the tumor, or at least we discovered it, on the second, or third, or possibly even later examinations. Therefore, we had a much better opportunity to discover tumors in this series of cases than would have been afforded by a single examination.

Chairman Brunschwig: Dr. Patterson.

Dr. Patterson: In answer to Dr. Schiff's question, I have not biopsied the normal appearing esophagus in an attempt to determine the limits of possible extension of carcinoma.

Dr. Schiff: That wasn't my point. The point I made was whether it would be feasible to take a piece of grossly normal esophagus for biopsy purposes.

Dr. Dunlap: I was interested in Dr. Gomori's remarks. Before we began these studies I went to see Dr. Gomori, and he showed me some of his data. It was not

too encouraging, but we went ahead anyway. Did I understand him to say he had not found more than 10 units per cc. or per 100 cc. in his studies?

Dr. Gomori: Per 100 cc.

Dr. Dunlap: We found a 70-percent loss of activity in samples that had stood at a room temperature of 90° for a few hours, and we now refrigerate the samples immediately on obtaining them.

Chairman Brunschwig: Dr. McNeer.

Dr. McNeer: There is another slant to Dr. Ivy's question about the extension of carcinoma above the lesion in the stomach. We found, so often, that it occurred submucosally in the esophagus as a metastasis; therefore, we would not expect to see it or to obtain biopsy specimens at esophagoscopy. Now, when resecting the gastric cardia we always subject the upper limits of the lesion to frozen section before proceeding with the anastomosis. We have, within the past year, had an instance of pancreatic cancer which was diagnosed preoperatively by cytological study of the duodenal secretions after the method of Papanicolaou.

Chairman Brunschwig: If no one else wishes to speak, we will now adjourn for the day.

(Whereupon, at 5:15 o'clock p. m. the conference adjourned, to reconvene at 9 o'clock a. m. the following day.)

TUESDAY MORNING SESSION
December 14, 1948

Gastric acidity in cases of adenomatous gastric polyp.....HORACE E. CROMER, MANDRED W. COMFORT, HUGH R. BUTT, and JOSEPH BERKSON

The topography of chronic gastritis in cancer-bearing stomachs.....ROBERT HEBBEL

Chronic atrophic gastritis: Identification of different varieties.....ALVIN J. COX

Malignancy of gastric cancer.....WILLIAM A. MEISSNER

The silent interval in gastric cancer: A study of the time elapsing before reassertion of symptoms in patients with microscopic malignancy in the proximal line of excision and without lymph-node metastases.....STANLEY R. FRIESEN

The Conference reconvened at 9:00 a. m., Tuesday morning, December 14, 1948, Dr. Walter L. Palmer, presiding.

Chairman Palmer: Gentlemen, it is time, I think, to start the morning session. The first paper is by Dr. Horace E. Cromer of Rochester, Minn., Gastric Acidity in Cases of Adenomatous Gastric Polyp. It will be presented by Dr. Comfort.

GASTRIC ACIDITY IN CASES OF ADENOMATOUS GASTRIC POLYP[1,2]

By HORACE E. CROMER, Jr., M. D., *Fellow in medicine, Mayo Foundation,* MANDRED W. COMFORT, M. D., and HUGH R. BUTT, M. D., *Division of Medicine, Mayo Clinic, Rochester, Minn.*

The pattern of secretion of free hydrochloric acid by precancerous and cancerous patients is becoming increasingly well defined. A subnormal mean secretory activity has been found characteristic of the precancerous patients regardless of the decade in life during which the initial test meal was ingested and analyzed and regardless of the interval in years between the initial test meal and the diagnosis of cancer. The subnormal mean secretory activity is more abnormal at the time when cancer is diagnosed than in the preceding years. At the time when cancer is diagnosed, achlorhydria is approximately 3 times as common as the normal in men and two and a half times as common in women, and the mean free acidity for men and women is, respectively, 14 and 8.7 clinical units less than the values expected in a group of normal persons of the same sex and age. The process responsible for lowering of the gastric acidity in gastric cancer may become active early in life and appears to be progressive and selective.

Because the adenomatous gastric polyp commonly is accepted as a precursor of gastric cancer, the questions well may be asked, "Is the pattern of gastric secretion by the patient with adenomatous gastric polyp similar to that of the patient with gastric cancer? Does adenomatous gastric polyp develop in a secretory environment similar to that in which gastric cancer develops? How well may the test meal be used as a screening test in picking out those patients in whom polyp will develop?"

Data bearing on gastric acidity in cases of adenomatous gastric polyp are relatively few. The series studied by Brunn and Pearl (*1*) in 1926, Miller, Eliason, and Wright (*2*) in 1930, Pearl and Brunn (*3*) in 1943 and Spriggs and Marxer (*4*) in 1943, as we interpreted the pathologic findings, include 59 cases of pathologically verified benign or malignant polyps in which acidity of the gastric contents was determined. Of the 59, there were only 5 cases (8.5 percent) in which free acid was found in the gastric contents. Of the 59, the polyps were benign in 22, benign but accompanied by a malignant lesion elsewhere in the stomach in 4, malignant in 29, and malignant but accompanied by a malignant lesion elsewhere in the stomach in 4. Free acidity was found in the gastric contents in 2 cases with benign polyp, in 2 cases with benign polyp accompanied by a malignant lesion elsewhere in the stomach, and in 1 case with malignant

[1] Read at the meeting of the Fourth Conference on Gastric Cancer, San Francisco, December 14, 1948.

[2] Abridgment of thesis submitted by Dr. Cromer to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M. S. in Medicine.

polyp. These data suggest that the gastric contents almost always are anacid when benign or malignant adenomatous polyp is present.

MATERIALS AND METHODS

In an attempt to secure more extensive data bearing on gastric acidity in adenomatous gastric polyp, we examined the records of all cases which were seen at the Mayo Clinic in which the adenomatous character of the polyp had been verified by microscopic examination. We selected for special study those 185 cases in which analysis of gastric contents had been performed. Of the 185 cases, the polyp was removed surgically in 168 and at necropsy in 17. In each of the 185 cases, the test meal was carried out at the time of the diagnosis. In 9 of the 185, another test meal had been performed 2 or more years prior to the time of the diagnosis. It is the data about gastric secretory activity obtained from the records of these 185 patients which we are presenting.

The test meal consisted of 8 arrowroot cookies and 400 cc. of water. After 1 hour, a 25-cc. sample of gastric contents was removed by means of a Sawyer tube. The sample was tested for free hydrochloric acid, and if this was present the stomach was emptied and the tube was withdrawn. If, on the other hand, there was no free hydrochloric acid present in the initial specimen, the tube was left in place, and additional samples were removed at intervals of 15 minutes. The results of titration are expressed in clinical units (Töpfer's method).

Data about gastric acidity obtained from the records of these groups of cases have been compared with similar data obtained by the same test meal in a series of normal persons of the same sex and age reported by Vanzant, Alvarez, Eusterman, Dunn, and Berkson (*5*) and in carcinomatous lesions, as reported by Comfort and Vanzant (*6*, *7*) and by Walters, Gray, and Priestley (*8*).

Of the 185 cases, in 150 the polyp was benign and in 35 malignant. Classifying the malignant process according to Broders' method, malignancy was graded 1 (malignancy in situ) in 27 cases and was graded 2, 3, or 4 in 8 cases. A single polyp was present in 98 cases, and multiple polyps were present in 83 cases. The lesion was classified as polyadenoma en nappe in the remaining 4 cases.

Intragastric lesions other than the benign or malignant polyp or polyps were noted. In 39 cases in which the polyp was benign, cancer also was present; in 19 of the 185 cases pernicious anemia was diagnosed. Of the 19, the polyp was benign in 9, malignant grade 1 (in situ) in 4, malignant grade 2, 3, or 4 in 2, and benign but associated with cancer elsewhere in the stomach in 4 cases. Most interesting was the associated finding of duodenal ulcer in at least 2, gastric ulcer in 4, gastrojejunal ulcer in 3, and severe, ulcerative gastritis in 3 cases. In several cases atrophic gastritis was recorded as being present, but undoubtedly this condition was not recorded in many cases in which it was present.

Of the 185 patients, 120 were male and 65 were female. The mean age and distribution by decades of life in relation to gastric acidity are shown in table 1.

TABLE 1.—*Adenomatous gastric polyp: gastric acidity in relation to age at diagnosis*

Pathological diagnosis	Cases with anacidity								Cases with free acidity								
	Cases	Age in years						Mean	Cases	Age in years						Mean
		20–29	30–39	40–49	50–59	60–69	70–79			10–19	20–29	30–39	40–49	50–59	60–69	70–79	
Total	156	1	5	23	54	59	14	57.1	29	1	0	6	12	6	3	1	47.2
Pathologically verified benign	95	1	3	12	37	36	6	57.2	16	0	0	5	8	2	1	0	42.8
Grade 1 malignant lesion in situ	24	0	1	5	3	12	3	58.8	3	1	0	0	1	0	1	0	42.3
Malignant grade 2, 3, or 4	7	0	1	2	3	1	0	52.4	1	0	0	0	0	1	0	0	51.4
Benign with malignant lesion elsewhere in stomach	30	0	0	4	11	10	5	59.4	9	0	0	1	3	3	1	1	52.3

GASTRIC ACIDITY AT THE TIME OF DIAGNOSIS OF ADENOMATOUS POLYP

Among the 185 patients with pathologically verified adenomatous gastric polyp for whom analysis of gastric contents was done at the time of diagnosis, 156 (84.3 percent) had achlorhydric gastric contents, and 29 (15.7 percent) had gastric contents containing free acid (table 2). In the 29 cases in which the gastric contents contained free acid, the mean free acidity was 26.8 clinical units (table 3). In short, among the group of 185 cases with both benign and malignant lesions, the incidence of achlorhydria was greater by 65.1 percent (table 2) than would be expected in a group of normal persons of the same sex and age, and the mean free acidity was subnormal by 15.9 clinical units (table 3). Moreover, regardless of the benignancy or malignancy of the adenomatous polyp or of associated malignant lesions, the percentage of anacidity was approximately four times the percentage of anacidity expected from a group of normal persons of the same sex and age (table 2). Also, the mean free acidity for the benign polyps and for cases of benign polyps associated with a malignant lesion elsewhere in the stomach was less than would be expected from a group of normal persons of the same age and sex. These data are similar to those obtained from cases recorded in the literature.

TABLE 2.—*Adenomatous gastric polyp: Incidence of achlorhydria*

Group	Cases	Cases with achlorhydria					
		Number	Percent	Mean age, years	Standard for age, percent	Difference from standard, percent	Standard deviation of difference
Pathologically verified	185	156	84.3	57.1	19.2	65.1	3.8
Benign	111	95	85.6	57.2	19.2	66.4	4.2
Grade 1 malignant lesion in situ	27	24	88.9	58.8	19.2	69.7	6.9
Grade 2, 3, or 4 malignant lesion	8	7	87.5	52.4	17.2	70.3	12.7
Benign with associated malignant lesion of stomach	39	30	76.9	59.4	19.2	57.7	8.1

Mean secretory activity in this series of benign and malignant polyps is more subnormal than in gastric carcinoma. Thus, the percentage of achlorhydria (84.3 percent) is markedly greater than the percentage of achlorhydria with the smallest gastric cancer (18 percent for lesions 3 by 3 cm. or less in diameter), much greater than with resectable gastric carcinoma (54 and 47 percent, respectively, in men and women or 51 percent for both sexes), and even greater than with nonresectable

TABLE 3.—*Adenomatous gastric polyp: Mean free acid compared with standard*

Group	Cases	Mean acid	Mean age, years	Standard acid for age	Mean difference observed from standard
Pathologically verified	29	26.8	47.1	42.7	−15.9±2.5
Benign	16	28.3	42.8	40.2	−11.9±4.1
Grade 1, malignant lesion in situ	3	40.0	42.3	40.2	+0.2
Grade 2, 3, or 4 malignant lesion	1	8.0	51.0	40.1	−32.1
Benign with associated malignant lesion of stomach	9	21.6	52.3	40.1	−18.5±3.2

lesions (72 and 62 percent, respectively, in men and women). Only when the gastric cancer is 7 by 7 cm. in diameter, or larger, does the percentage of achlorhydria (80 to 90 percent) equal the percentage seen in the group with adenomatous polyp. It has been suggested that the loss of secretory activity of the cancerous stomach might be due partly to destruction of the mucous membrane by the cancer, but in the group of polyps, most of which are small and single, the loss cannot be attributed to this cause.

The distribution of free acid in units is shown in table 4. All degrees of free acidity up to 60 to 69 clinical units were encountered in the gastric contents of patients with adenomatous gastric polyps, as is the case in carcinoma of the stomach.

TABLE 4.—*Adenomatous gastric polyp: Free acidity in units*

Group	Cases [1]	Units of free acid							
		1–9	10–19	20–29	30–39	40–49	50–59	60–69	Mean
Pathologically verified	29	4	4	6	6	6	1	2	26.8
Benign	16	2	2	4	4	3	0	1	28.3
Grade 1 malignant lesion in situ	3	0	0	0	1	2	0	0	40.0
Grade 2, 3, or 4 malignant lesion	1	1	0	0	0	0	0	0	8.0
Benign with associated malignant lesion of stomach	9	1	2	2	1	1	1	1	21.6

[1] Only cases showing free gastric acidity are included.

GASTRIC SECRETORY ACTIVITY BEFORE DEVELOPMENT OF THE ADENOMATOUS GASTRIC POLYP

In 9 of the 185 cases of adenomatous gastric polyp (table 5) a test meal was performed for the patient 2 to 24 years and at a mean interval of 11.7 years before the diagnosis was established by roentgenologic examination or by surgical resection. In 6 of the 9 cases the roentgenologists reported

that they could not demonstrate an intragastric lesion at the time of the original test meal. In 3 of the 9 cases a roentgenologic examination was not carried out at the time when the original analysis of gastric contents was performed for the patient 13, 17, and 24 years, respectively, before the diagnosis was established. In 7 of the 9 cases, gastric contents were achlorhydric both at the time of the original test meal and at the time of the test meal performed for the patient when the diagnosis was established. In the remaining 2 cases, the gastric contents contained free hydrochloric acid at the time of the original test meal but had become anacid 13 and 24 years later, respectively, when the diagnosis of adenomatous gastric polyp was established.

TABLE 5.—*Adenomatous gastric polyp: Gastric acidity before and after development*

Case	At time of first examination			Interval between test meals, years	At time of diagnosis		
	Age, years	Free acidity, units	Roentgenologic diagnosis		Free acidity units	Roentgenologic diagnosis [1]	Pathologic diagnosis [2]
1	48	0	Negative	11	0	P	AP.
2	38	0	Negative	14	0	MP	MAP (3).
3	28	0	Negative	17	0	MP	MAP.
4	59	0	Negative	6	0	P	AP.
5	54	0	Negative	2	0	PL	X.
6	52	0	Negative	2	0	P	AP.
7	31	0		17	0	P	AP.
8	43	16		24	0	MP	MAP (20).
9	51	36		13	0	PL	AP.

[1] P=polyp; MP=multiple polyps; PL=polypoid lesion.
[2] AP=adenomatous polyp; MAP=multiple adenomatous polyps; X=adenocarcinoma, grade 1, in adenomatous polyp.

In a recently studied series of 277 cases of gastric cancer in which analysis of gastric contents was performed once or more, 2 or more years before gastric cancer was diagnosed, Comfort, Kelsey, and Berkson (*9*) found that gastric cancer appeared to develop in stomachs of lower than mean secretory activity. The number of cases of adenomatous gastric polyp in the present paper in which analysis of gastric contents was performed 2 or more years before the diagnosis was made is too small for detailed study as carried out by these authors or for general conclusions, but it appears that the mean secretory activity of the stomach destined to become the site of an adenomatous polyp was subnormal probably long before the polyp developed in these 9 cases.

SUMMARY AND CONCLUSIONS

In a series of 185 cases of pathologically verified adenomatous gastric polyp, mean gastric secretory activity was subnormal. The incidence of achlorhydria was greater by 65.1 percent and the mean free acidity was less by 15.9 clinical units than would be expected in a group of normal persons of the same sex and age.

The mean secretory activity was lower than would be expected in a group of normal persons regardless of the benignancy or malignancy of the polyp, or of a malignant lesion elsewhere in the stomach.

The mean secretory activity was more subnormal than with resectable and even nonresectable cancerous lesions of the stomach. Only when gastric cancer was large or inoperable did the mean gastric secretory activity approach that of adenomatous polyp.

In 9 of the 185 cases in which analysis of gastric contents was performed 2 or more years before adenomatous gastric polyp was diagnosed, the mean secretory activity had been subnormal at a mean interval of 11.7 years before the diagnosis of polyp was made, and was subnormal probably before the polyp developed, just as in gastric cancer.

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Chairman Palmer: Thank you, Dr. Comfort. It has been suggested that we defer discussion on this paper and on the next paper until after the third paper of Dr. Cox. The second paper will be by Dr. Robert Hebbel of Minneapolis; it is entitled The Topography of Chronic Gastritis in Cancer-Bearing Stomachs. Dr. Hebbel.

THE TOPOGRAPHY OF CHRONIC GASTRITIS IN CANCER-BEARING STOMACHS[1,2]

By ROBERT HEBBEL, M. D., *Department of Pathology, University of Minnesota, Minneapolis*

The significance of the frequent association of chronic gastrities with carcinoma of the stomach has been extensively discussed in the literature. In brief, opinions favoring a causal relationship between gastritis and carcinoma are based largely on the frequency of the association (Konjetzny (*1*), Orator (*2*), Hurst (*3*) and others) and the demonstration of histological features interpreted as evidence of transitions between altered mucosas and carcinoma (Konjetzny (*1*)). Opposition to this view is founded on the frequency with which gastritis is encountered in older persons (Hillenbrand (*4*), Guiss and Stewart (*5*) and others) the inconstancy of the association of extensive gastritis and carcinoma (Hebbel (*6*), Magnus (*7*) and others) and the absence of gastritis in cases of early carcinoma (Borrmann (*8*)). Cancer gastritis has often been described as a pangastritis (Konjetzny (*1*), Orator (*2*), Tuomikoski (*9*) and others) for the great majority of cases. Exceptions have been noted, but the exact frequency of diffuse gastritis with carcinoma of the stomach is not entirely well defined. If chronic gastritis of a diffuse character is important, it should be present in a significant majority of the cases of gastric cancer. It seemed desirable to provide a more accurate picture of gastritis with cancer with particular reference to the incidence of diffuse changes based on a series of cases large enough to at least minimize sampling errors.

MATERIAL AND METHODS

This study is based on 199 resected carcinoma-bearing stomachs. Except for a very few total gastrectomies, the specimens represent subtotal resections. Table 1 summarizes the material with respect to age, sex, and gross type (Borrmann (*10*)) of tumor. The cases are selected only with respect to the availability of reasonably large segments of mucosa uninvolved by tumor on at least one curvature of antrum or body. Instances in which the entire, or most, of the resected segment was occupied by tumor and specimens which had been badly mutilated were eliminated.

The sizes of the excised segments of stomach and consequently the lengths of the curvatures varied. However, since the resections represented, for the most part, comparable proportions of the whole organ, the individual segments of antrum and body, though differing in actual length, are roughly comparable.

[1] Presented at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 14, 1948.

[2] Aided by a grant from the National Advisory Cancer Council, U. S. Public Health Service.

TABLE 1.—*Age and sex distribution of 199 resected gastric carcinomas with respect to the gross type (Borrmann) of the tumors*

Type of carcinoma (Borrmann)	Males		Females		Total
	Over age 50	Under age 51	Over age 50	Under age 51	
I	2	1	2		5
II	41	5	17		63
III	25	4	4		33
IV	61	10	23	4	98
Total	129	20	46	4	199

In each specimen, strips of mucosa were excised from the available portions of lesser and greater curvatures respectively. These strips were rolled up, blocked, and sectioned. The size and location of the tumor determined the source and length of the preparations in any one specimen. Both curvatures of the antrum and the lesser curvature of the body were less frequently available than was the greater curvature of the body. In but few specimens was the whole of one or both curvatures available. In each strip the proportion of the excised segment of antrum or body represented was estimated and, roughly indicating thirds, was recorded as 1, 2, or 3. Thus, if a carcinoma involved a third of the lesser curvature of the antrum, the designation for the part examined was 2.

The sections were searched for mucosal abnormalities. The findings relative to the presence, severity, and distribution of cellular infiltration of the stroma, lymphoid nodules, atrophy, metaplasia, and pseudopyloric glands were recorded. Other less frequent accompanying alterations such as scarring, cystic changes, and erosions have been omitted from the tabulations. Each of the several categories was graded, when present, on a basis of 1 to 3 representing mild, moderate, and severe degrees respectively.

Cellular infiltration of the stroma was considered to be absent when the usually encountered lymphocytes and plasma cells were very few and inconspicuous. Grade 1 infiltration represents small numbers of such cells confined chiefly to the interfoveolar stroma. Grade 2 infiltration indicates a moderate number in the same areas or sometimes extending more deeply. Grade 3 infiltration represents a heavy concentration of lymphoid cells of obviously abnormal degree. Cellular infiltration was rather uniform throughout the segment concerned. It may be noted here that no specimen without parenchymal changes showed an unquestionably significant degree of cellular infiltration.

Lymph nodules, with or without reaction centers, were graded 1 to 3. Grade 1 indicates 1 to 5 nodules, grade 2 indicates 6 to 12 nodules, and grade 3 indicates a greater number for the segment concerned. No specimen was called abnormal because of lymph nodules alone.

Atrophy was graded 1 to 3. The term is here used to represent loss of normal glandular structure with or without actual shortening or disappearance of the glands and the consequent thinning of the mucosa. Atrophy is frequently paralleled by metaplasia or pseudopyloric glands or both. Grades 1 and 2 indicate partial loss of gland structure of mild and moderate degrees while grade 3 indicates complete or almost complete absence of normal cell pattern. The tabulations show atrophy in terms of severity for the area involved whether focal or diffuse and the distribution of the atrophy is given in the indicated extent of the process.

Metaplasia was graded 1 to 3 to indicate mild, moderate, and severe degrees of intestinalization.

Pseudopyloric glands in the body mucosa were similarly graded 1 to 3 and alone or with metaplasia often parallel atrophy in both severity and distribution.

On the basis of the above findings, the specimens were classified as normal when free of change or abnormal when parenchymal changes were present. The distributions of the changes were noted to be focal, patchy, partial (body only), or diffuse. Those classed as focal are specimens which showed one to several minute, microscopic foci of atrophy usually with metaplasia or pseudopyloric glands. Those classed as patchy are specimens which showed one to several somewhat larger areas of similar change.

Partial involvement refers to the presence of uniformly diffuse changes in a portion of the examined segment of body mucosa. Comparable distributions were not observed in the antrum. It has been convenient to designate these partial involvements as ⅓ and ⅔ to roughly indicate the proportion of the segment so affected. Some of these may represent the involvement of the lower body commonly associated with a diffuse antral gastritis, but others occur in the absence of antral changes. The exact limits are, of course, difficult to define, for these processes encroach on the transition zone where, under normal circumstances, the dividing line between antrum and body may not be sharp. Many of these classed as ⅓ are not quantitatively significant, but they are more extensive than patchy lesions and consequently were given a separate designation. In some instances of a tumor at the cardia, comparable limited changes involved that portion of the body mucosa bordering the tumor while the lower body was normal.

Specimens which showed changes throughout were classified as diffuse, and the severity of the process was noted to be mild, moderate, or severe. Diffuse changes were quite uniform throughout. In a few specimens, the changes decreased in intensity as the line of resection was approached.

The rolls conveniently show the extent of the changes encountered. Focal and small patchy lesions may of course be missed. In either antrum or body the entire lesser curvature may be abnormal without comparable changes elsewhere. In such instances, the changes appear to be confined rather closely to the lesser curvature. Abnormality throughout the greater curvature of either antrum or body is indicative of a diffuse process

throughout the segment concerned. Under such circumstances preparations from other areas such as the anterior and posterior walls show comparable changes.

PRESENTATION OF DATA

Table 2 summarizes the findings on the lesser curvature of the antrum in 62 cases with respect to age and type of tumor. In 42 specimens the whole segment was examined, in 16 specimens ⅔ of the segment was examined, and in 4 specimens but ⅓ of the segment was examined. Infiltration was present in 61 specimens, of which 16 were of grade 1 and 45 were of grade 2. Follicles were noted in 58 specimens: 14 grade 1; 38 grade 2; and 6 grade 3. Fifty-four specimens showed atrophy: 19 grade 1; 17 grade 2; 18 grade 3. Fifty-one specimens showed metaplasia: 20 grade 1; 15 grade 2; 16 grade 3. In summary, 8 specimens were completely normal, 9 showed focal changes, 2 showed patchy changes, and the changes were diffuse in 43 of which 10 were mild, 13 moderate, and 20 severe.

Table 3 gives the findings on the greater curvature of the antrum in 93 cases. The data are presented in the same manner as for the lesser curvature, and reference may be made to the table for the details. In summary, 19 specimens were entirely normal, 25 showed focal lesions, 6 showed patchy lesions, and 43 showed diffuse changes of which 6 were mild, 10 moderate, and 27 severe.

Table 4 summarizes the findings on the lesser curvature of the body in 85 cases. The whole of the resected segment was examined in 73 cases, ⅔ was examined in 5 cases, and ⅓ was examined in 7 cases. The findings with regard to infiltration, lymph follicles, atrophy, and metaplasia are tabulated as they were for the antrum. This table also shows pseudopyloric glands and, in the classification, the category designated as partial. The lesser curvature of the body was completely normal in 15 specimens. Two showed focal changes, 2 showed uniform involvement of but about ⅓ of the segment, and 2 showed a similar involvement of about ⅔ of the segment while the remaining portions were free of change. (The whole segment was examined in these cases.) Sixty-four specimens showed diffuse changes, and among these the changes were mild in 4, moderate in 8, and severe in 52.

Table 5 summarizes the findings on the greater curvature of the body in 196 cases. Examination was limited to ⅓ of the segment in 5 cases, to ⅔ of the segment in 24 cases, and included the whole segment in 167 cases. The several degrees of infiltration, follicles, atrophy, metaplasia, and pseudopyloric glands may be noted in the table. The classification shows that 38 specimens were entirely normal, and 4 showed but focal changes. Extensive changes involved ⅓ of the segment in 31 cases and ⅔ of the segment in 14 cases. Changes were present throughout the segment examined in 109 specimens and were mild in 10, moderate in 24, and severe in 75.

TABLE 2.—*Summary of findings on lesser curvature antrum in 62 cases with respect to age and type of tumor*

Age	Type of tumor	Portion of segment examined			Infiltration			Follicles			Atrophy			Metaplasia			Classification						Total cases
																	Normal	Focal	Patchy	Diffuse			
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3				Mild	Moderate	Severe	
Under age 51	I			1		1			1			1			1						1		1
	II		1	2	3			1	1				1		1		2					1	3
	III			1		1			1		1			1				1					1
	IV	1		7		7			3	3	3	2	2	3	1	2	1	1		2	2	2	8
Total		1	1	11	3	9		1	6	3	4	3	3	4	3	2	3	2		2	3	3	13
Over age 50	I			4	2	2		2	2		1		2		1	1	1			1		2	4
	II	2	3	11	7	9		5	10	1	5	6	4	5	5	5	1	3	1	2	3	6	16
	III	1	3	7	1	10		1	9	1	4	5	2	6	3	2		2	1	2	4	2	11
	IV		9	9	3	15		5	11	1	5	3	7	5	3	6	3	2		3	3	7	18
Total		3	15	31	13	36		13	32	3	15	14	15	16	12	14	5	7	2	8	10	17	49
All cases	I			5	2	3		2	3		1	1	2		2	1	1			1	1	2	5
	II	2	4	13	10	9		6	11	1	5	6	5	5	6	5	3	3	1	2	3	7	19
	III	1	3	8	1	11		1	10	1	5	5	2	7	3	2		3	1	2	4	2	12
	IV	1	9	16	3	22		5	14	4	8	5	9	8	4	8	4	3		5	5	9	26
Total		4	16	42	16	45		14	38	6	19	17	18	20	15	16	8	9	2	10	13	20	62

TABLE 3.—*Summary of findings on greater curvature of antrum in 93 cases*

Age	Type of tumor	segment examined			Infiltration			Follicles			Atrophy			Metaplasia			Classification						Total cases
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Normal	Focal	Patchy	Diffuse			
																				Mild	Moderate	Severe	
Under age 51	I			1		1			1		1			1				1					1
	II			1	2	2		1	2		2	1		2	1		1	1	2				4
	III			3	1	2			3		1			1			2	1					3
	IV		1	4		4		1	2	1	2		1	2		1	2	1		1		1	5
Total		2	2	9	3	9		2	8	1	6	1	1	6	1	1	5	4	2	1		1	13
Over age 50	I			3	2	1		2	1		1		1	1		1	1	1				1	3
	II	3	5	19	9	17		8	14	4	11	4	6	9	7	4	6	8	3		3	7	27
	III	2	1	14	3	14		3	14		6	4	5	8	3	4	2	5		1	3	6	17
	IV	2	11	20	3	30		5	27	1	11	10	7	11	10	7	5	7	1	4	4	12	33
Total		7	17	56	17	62		18	56	5	29	18	19	29	20	16	14	21	4	5	10	26	80
All cases	I			4	2	2		2	2		1		1	1		1	1	2				1	4
	II	5	6	20	11	19		9	16	4	13	5	6	11	8	4	7	9	5		3	7	31
	III	2	1	17	4	16		3	17		7	4	5	9	3	4	4	6		1	3	6	20
	IV	2	12	24	3	34		6	29	2	13	10	8	13	10	8	7	8	1	5	4	13	38
Total		9	19	65	20	71		20	64	6	34	19	20	34	21	17	19	25	6	6	10	27	93

TABLE 4.—*Summary of findings in the lesser curvature of the body in 85 cases*

Age	Type of tumor	Portion of segment examined			Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Classification							Total cases
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Normal	Focal	Partial		Diffuse			
																						½	¾	Mild	Moderate	Severe	
Under age 50	I	1				1			1			1			1			1									1
	II			3		3			3		1	2	1	2				3							1	2	3
	III			2		2			2					2		1	1	2					1		1		2
	IV		1	3	1	1		1	1					1			1	1		3						1	4
Total		1	1	8	1	7		1	7			2	5	1	4	2		7		3			1		3	3	10
Over age 50	I	1		3	1	3			3				4	1	3			4								4	4
	II	2	1	17	3	16	1	3	14	2	5		15	3	7	8	9	10			2			3	1	14	20
	III	1	2	10	4	7	1	2	8	1			9		4	5	4	3	2	4						9	13
	IV	2	1	35	9	27	1	8	24	2	2	4	24	2	19	8	6	21	1	8		2	1	1	4	22	39
Total		6	4	65	17	53	3	13	49	5	7	4	52	6	33	21	19	38	3	12	2	2	1	4	5	49	75
All cases	I	2		3	1	4			4			1	4	1	4			5							1	4	5
	II	2	1	20	3	19	1	3	17	2	5	1	17	4	9	8	9	13			2			3	2	16	23
	III	1	2	12	4	9	1	2	10	1			11		5	6	4	5	2	4			1		1	9	15
	IV	2	2	38	10	28	1	9	25	2	2	4	25	2	19	9	6	22	1	11		2	1	1	4	23	42
Total		7	5	73	18	60	3	14	56	5	7	6	57	7	37	23	19	45	3	15	2	2	2	4	8	52	85

TABLE 5.—*Summary of findings on greater curvature of the body in 196 cases*

Age	Type of tumor	Portion of segment examined			Infiltration			Follicles			Atrophy			Metaplasia			Pseudopyloric glands			Classification							Total cases
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Normal	Focal	Partial		Diffuse			
																						⅓	⅔	Mild	Moderate	Severe	
Under age 50	I	--	--	1	--	1	--	--	--	1	--	1	--	--	1	--	--	1	--	------	------	----	----	------	1	------	1
	II	--	1	4	1	4	--	1	4	--	--	2	2	1	3	--	--	4	--	1	------	----	1	------	2	1	5
	III	--	--	4	2	2	--	2	2	--	1	--	1	1	1	--	1	1	--	2	------	2	----	------	------	------	4
	IV	--	3	10	5	4	1	2	6	0	2	2	2	1	--	2	1	5	--	7	------	1	----	------	2	3	13
Total		--	4	19	8	11	1	5	12	1	3	5	5	3	5	2	2	11	--	10	------	3	1	------	5	4	23
Over Age 50	I	--	2	2	1	3	--	--	4	--	--	--	4	--	4	--	--	4	--	------	------	----	----	------	------	4	4
	II	1	7	50	3	52	1	8	39	7	6	17	32	8	36	10	6	44	6	3	2	10	4	4	9	26	58
	III	--	2	27	3	22	2	3	21	3	1	6	16	2	7	13	4	14	5	6	------	4	1	1	3	14	29
	IV	4	9	69	15	63	2	13	59	4	8	18	36	13	33	14	9	41	12	19	2	14	8	5	7	27	82
Total		5	20	148	22	140	5	24	123	14	15	41	88	23	80	37	19	103	23	28	4	28	13	10	19	71	173
All Cases	I	--	2	3	1	4	--	--	4	1	--	1	4	--	5	--	--	5	--	------	------	----	----	------	1	4	5
	II	1	8	54	4	56	1	9	43	7	6	19	34	9	39	10	6	48	6	4	2	10	5	4	11	27	63
	III	--	2	31	5	24	2	5	23	3	2	6	17	3	8	13	5	15	5	8	------	6	1	1	3	14	33
	IV	4	12	79	20	67	3	15	65	4	10	20	38	14	33	16	10	46	12	26	2	15	8	5	9	30	95
Total		5	24	167	30	151	6	29	135	15	18	46	93	26	85	39	21	114	23	38	4	31	14	10	24	75	196

Table 6 shows the distribution of the changes in 102 specimens in which portions of both antrum and body were available for examination. In this and subsequent tables, focal and patchy lesions have been combined with the entirely normal group. Only those specimens which presented diffuse changes throughout the segment of antrum or throughout a part or the whole of the segment of body mucosa are listed as abnormal. Both curvatures of the antrum were examined in 49 specimens, the lesser curvature alone was examined in 10 specimens, and only the greater curvature was examined in 43 specimens. Both curvatures of the body were examined in 53 specimens, and only the greater curvature was examined in 49 specimens. The varying combinations in the distribution of the findings are shown. Thus, diffuse changes may be present on the lesser or both curvatures of the body when the antrum shows no significant change or is but partially involved, the reverse may obtain, or diffuse changes may be present in both. For example, of the 19 specimens which showed diffuse changes on both curvatures of the antrum, both curvatures of the body were examined in 15 instances. Eight showed diffuse changes on both curvatures, in 4 less extensive changes were found, and in 3 both curvatures were normal. Similarly, both curvatures of the body showed diffuse changes in 27 specimens. In 21 of these specimens both curvatures of the antrum were examined, and of these 5 were normal on both curvatures, 8 showed changes on the lesser curvature only, and 8 showed diffuse changes on both curvatures. Reference to the table will disclose other combinations.

Table 7 summarizes the findings in the antrum with reference to type, size, and location of the tumor in 105 cases in which one or both curvatures were examined. Location indicates whether the tumor was confined to the antrum, involved both antrum and body, or was confined to the body. Of the 50 cases in which both curvatures were examined, 20 (40 percent) showed diffuse changes on both curvatures. One may with confidence presume that diffuse changes on the greater curvature are indicative of diffuse changes throughout the segment. For the entire group, as indicated by the findings on the greater curvature, 43 of 105 cases showed diffuse changes, again about 40 percent. Proportions of about the same magnitude are found in reference to the several gross types of tumor except in the case of type I where the number is too small to form a basis for calculation. No striking differences are assignable to the effects of location or size of the tumor within the several types. What little evidence of difference exists is seen in relation to the location of tumors in the body mucosa with which cases there are fewer instances of diffuse antral gastritis.

TABLE 6.—*Distribution of gastritic changes in 102 cases in which portions of both antrum and body were examined*

Body	Cases	Findings in antrum						
		Both curvatures examined			One curvature examined			
					Lesser		Greater	
		Both normal	Only lesser abnormal	Both abnormal	Normal	Abnormal	Normal	Abnormal
	Number							
Both curvatures examined	53							
Both normal	9	2	1	3		1		2
Only lesser abnormal:								
Partial	1							1
Diffuse	4	1		2				1
Only greater abnormal, all partial	4	1		1				2
Diffuse lesser, partial, greater	8	3	1	1		1	1	1
Both diffuse	27	5	8	8		4	1	1
Only greater examined	49							
Normal	14		1	2	2		7	2
Partial	12	1	1	2			4	4
Diffuse	23	2	3			2	7	9
Totals	102	15	15	19	2	8	20	23

The findings in the body mucosa relative to type, size, and location of the tumor are consolidated in table 8 for 196 cases in which one or both curvatures were examined. Reference may be made to the table for the details. Both curvatures were examined in 85 cases, and the greater curvature only was examined in 111 cases. Of those in which both curvatures were examined, 12 were normal, 6 showed changes (1 partial, 5 diffuse) on the lesser curvature only, 5 showed partial involvement of the greater curvature only, 3 showed partial involvement of both curvatures, 11 showed diffuse changes on the lesser and partial involvement of the greater curvature, and 48 showed diffuse changes on both curvatures. Of the 111 specimens in which only the greater curvature was examined, 24 were normal, 26 showed partial involvement, and 61 showed diffuse

TABLE 7.—*Summary of findings in antral mucosa in 105 cases with respect to type, size, and location of carcinoma*

	Both curvatures examined			One curvature examined				Total cases
				Lesser		Greater		
	Both normal	Only lesser abnormal	Both diffuse	Normal	Diffuse	Normal	Diffuse	
A. Type of tumor:								
I	1	2	1	------	1	------	------	5
II	7	7	3	------	2	7	7	33
III	4	2	5	------	1	4	5	21
IV	3	4	11	4	4	9	11	46
Total cases	15	15	20	4	8	20	23	105
B. Size of tumor:								
Up to 5 cm. in diameter	4	12	14	------	------	6	9	45
Over 5 cm. in diameter	11	3	6	4	8	14	14	60
Total	15	15	20	4	8	20	23	105
C. Location of tumor:								
Antrum (II–17, III–6, IV–25)	3	8	9	------	5	9	14	48
Antrum and body (I–1, II–9, III–8, IV–8)	2	0	3	1	1	10	9	26
Body (I–4, II–7, III–7, IV–13)	10	7	8	3	2	1	------	31
Total cases	15	15	20	4	8	20	23	105

changes. Here again, considerable variation in the distribution of changes is encountered. As in the case of the antrum, diffuse changes along the greater curvature of the body are indicative of diffuse changes throughout that segment. Considering only the greater curvature, it may be seen in table 8 that there were no significant changes in 42 specimens, and changes were diffuse in 109 specimens. Thus, 21 percent are normal, 23 percent show partial involvement, and 56 percent show diffuse changes. Location and size of the tumor exert no particular influence on the distribution of the changes in the body mucosa. Diffuse changes were noted in all of the cases of Type I carcinomas. Such diffuse gastritis appears to be commonly associated with polypoid carcinomas. Cases with tumors of Type IV (about half of the total) provide about two-thirds of the normal mucosas.

TABLE 8.—*Summary of findings in body mucosa in 196 cases with respect to type, size, and location of the tumor*

	Both curvatures examined								One curvature examined			Total cases
	Both normal	One abnormal				Both abnormal			Greater			
		Lesser		Greater		Both partial	Diffuse lesser partial greater	Both diffuse	Normal	Partial	Diffuse	
		Partial	Diffuse	Partial	Diffuse							
A. Type of tumor:												
I								5				5
II	1		1	1			4	16	4	10	26	63
III	4		1			1	2	7	3	4	11	33
IV	7	1	3	4		2	5	20	17	12	24	95
Totals	12	1	5	5		3	11	48	24	26	61	196
B. Size of tumor:												
Up to 5 cm	4	1	3	3		1	4	18	10	6	12	62
Over 5 cm	8		2	2		2	7	30	14	20	49	134
Totals	12	1	5	5		3	11	48	24	26	61	196
C. Location of tumor:												
Antrum	10	1	4	5		3	8	35	6	9	25	106
Antrum and body							2	2	10	11	27	52
Body	2		1				1	11	8	6	9	38
Total	12	1	5	5		3	11	48	24	26	61	196

COMMENT

The above data show that while gastritis is found in most carcinoma-bearing stomachs, there are wide variations in the severity and distribution of the mucosal changes. There is no constancy in the pattern of involvement of the antrum and body of the same stomach. In either segment, the gastritic changes range from focal to diffuse in distribution. Few cases are free of focal lesions, and it is likely that were a large enough number of sections examined, none would be completely free. Gastritic changes may involve the immediate region of the lesser curvature without comparable changes elsewhere, but diffuse changes along the entire greater curvature occur only in association with diffuse changes through the whole of the segment concerned. There is perhaps no sharp dividing line between insignificant and significant changes in quantitative terms, but diffuse involvement may certainly be considered distinctly abnormal. As measured by the changes on the greater curvature, the antral mucosa showed diffuse gastritis in about 45 percent of those specimens examined, and the body mucosa showed diffuse gastritis in about 55 percent of the specimens examined.

It is of interest to compare the findings in this group of cases with the findings in the stomachs of persons free of manifest gastric disease. Reported incidences of gastritis vary with the type and extent of examinations as well as with varying concepts of abnormality. Sharp distinctions have not always been made between diffuse and less extensive changes. Most observers agree that gastritis is common, particularly in older age groups. Including abnormalities of all degrees such is the case in my own material (*6*, *11*). In a series of 97 stomachs (*11*) from individuals of all ages, 72 percent showed some type of abnormality. Diffuse parenchymal changes of the sort referred to in the carcinoma-bearing stomachs were present in the antrum in 13 percent and in the body in 20 percent of the 53 stomachs from persons over 50 years of age. A larger material would possibly modify the percentages, but it is believed that while the incidence of diffuse changes might be a little greater, it would not be lower.

The general patterns of distribution of gastritic changes are the same in stomachs with and without carcinoma. The only difference lies in the partial involvement of the body mucosa in some cases in the cancer group. In a few instances, diffuse changes were of decreasing severity as the line of resection was approached. It is also true that for these resected stomachs the character of the residual pouch is not known. Since, in some instances, the entire resected segment is not involved, it is possible that changes throughout the resected segment may not always reflect the character of the pouch. Distributions of parenchymal changes comparable to the above partial involvement of the body mucosa have not been observed in my noncancer material where diffuse gastritis, when present in the body mucosa, is of striking uniformity throughout. It is possible, of course, that an insufficient experience accounts for their absence. Whether the several degrees of partial involvement of the body mucosa observed in cancer-bearing stomachs is evidence for an upward-spreading

gastritis is uncertain. If so, one would also have to include a downward-spreading gastritis as reflected by partial involvement of the proximal body mucosa in relation to cardiac tumors.

It is obvious that in a material of this kind few, if any, cases permit an inference as to the character of the mucosa at the site of origin of a tumor. For example, in the case of a carcinoma occupying the entire antrum with an associated diffuse gastritis in the body mucosa, one does not know the former character of the antrum. It may have been normal, or it may have showed localized or diffuse changes. In the case of a tumor confined to the lesser curvature of an antrum in which the greater curvature shows no changes, one does not know that the lesser curvature was previously normal. On the other hand, with an antral tumor confined to the lesser curvature, diffuse gastritis on the greater curvature is evidence that the tumor began in an abnormal mucosa provided that the observed changes preceded the tumor. Similar examples involving other areas can be cited.

Focal and small patchy lesions are so common in all stomachs, particularly along the lesser curvature that little importance can be attached to them. Does the presence of diffuse gastritis have a greater significance in relation to gastric carcinoma? In the material reviewed here, diffuse mucosal changes in the antrum or body are independent of each other and of the size and location of the tumor. The process varies in severity and ranges, particularly in the body mucosa, from very mild alterations to the severe atrophy of the several included cases of pernicious anemia. While we are concerned here chiefly with topography, it is pertinent to make reference to certain histological details. In instances of comparable severity as measured by loss of specific glandular structure, there may be considerable difference in the qualitative features. One case may present a thin atrophic mucosa like that in pernicious anemia, another may show an equally severe reduction in specific glandular elements but the mucosa is of normal thickness and quite regularly arranged glands are lined by indifferent or metaplastic epithelium. Such variations suggest processes of unequal duration. Consequently, among the cases of comparable severity, one cannot assume that the changes are of equal chronicity.

This material shows an incidence of diffuse gastritis which is considerably higher for both body and antrum than that found in my series of autopsy cases, and the changes are more frequently severe. The frequency with which diffuse changes are encountered in persons over 50 years of age is such that the association should be present in a considerable number of cases on that basis alone. It does not necessarily follow that the difference is accounted for by a greater contribution to cases of carcinoma by the proportion of persons with an established gastritis. The possible limits of time required for the evolution of diffuse gastritis are not known, and it seems likely that some of the observed changes may be of short duration. Similarly, the possible limits of time concerned in the evolution of a carcinoma prior to the onset of symptoms are not known though there is evidence that it may be very long. Gastritis does not always accompany carcinoma, and when the association exists, it does not

seem necessary that the gastritis always precede the carcinoma. That diffuse as well as local changes may develop in the presence of a carcinoma is certainly possible. The mere presence of a carcinoma cannot be considered a cause of gastritis, and whether a carcinoma might contribute to circumstances responsible for a diffuse gastritis is uncertain. However, even granting that in all instances of carcinoma with diffuse gastritis, the mucosal changes preceded the appearance of the tumor, the association occurs in only about half of the cases. This proportion is not impressive, and such findings do not support a causal relationship between diffuse gastritis and carcinoma of the stomach.

It is of further interest to consider the degree to which these observed mucosal changes can be correlated with the secretory function of the stomach in the presence of carcinoma. Gastric acidities after histamine stimulation were determined in 173 cases. These data are collected in table 9 in reference to the findings on the greater curvature of the body.

TABLE 9.—*Mucosal changes on greater curvature of body in relation to gastric acidity in 173 cases*

	Normal	Gastritis					Total cases	
		Partial		Diffuse				
		One-third	Two-thirds	Mild	Moderate	Severe	Number	Percent
Achlorhydria	5	8	7	10	27	53	110	63
Free acid under 20°	5	4	1	1	6	0	17	10
Free acid over 20°	22	17	3	1	3	0	46	27
Total	32	29	11	12	36	53	173	------

Of the entire group 63 percent showed achlorhydria, 17 percent had less than 20 degrees of free acid, and 27 percent had more than 20 degrees of free acid. In general table 9 shows a higher incidence of achlorhydria with increasingly severe diffuse gastritis and the reverse obtains. However, there are both achlorhydria and low acid values in some instances of normal mucosas, and normal acidity is found in association with diffuse gastritis of less than severe degree. Only those with severe diffuse changes are constantly achlorhydric, and this group of 53 cases makes up about half of those with achlorhydria and a third of the total cases. The findings further serve to show that gastritis involving only the juxta-antral portion of the body mucosa is not quantitatively significant. This group of 173 cases includes a considerable number of tumors which involved more or less of the body mucosa, and some replaced a large portion of its total area. Table 10 shows the relationship between the degree of gastritis in the body mucosa and acidity in 89 cases in which the tumor was confined to the antrum. In each of these cases the entire greater curvature of excised body mucosa was examined. The findings

are about the same. Fifty-five percent showed achlorhydria, 11 percent showed less than 20 degrees, and 34 percent showed more than 20 degrees of free acid. As before, only those cases with severe diffuse gastritis constantly had achlorhydria, and this group, about a third of the total cases, provides about 60 percent of the cases with achlorhydria.

TABLE 10.—*Mucosal changes on greater curvature of the body in relation to gastric acidity in 89 cases in which the tumor [1] was confined to the antrum*

	Normal	Gastritis					Total cases	
		Partial		Diffuse				
		One-Third	Two-Thirds	Mild	Moderate	Severe	Number	Percent
Achlorhydria	2	3	1	3	10	30	49	55
Free acid under 20°	2	2	1	1	4	0	10	11
Free acid over 20°	12	12	2	1	3	0	30	34
Total	16	17	4	5	17	30	89	

[1] Type II—24; Type III—12; Type IV—53.

Achlorhydria can be accounted for on the basis of loss of acid cells in about half of the cases. Diffuse gastritis in the body mucosa is more likely to be associated with achlorhydria, but only in the presence of severe changes can one with reasonable certainty say that achlorhydria must have been present. It was pointed out above that chronic gastritis is of uncertain and probably varying duration. To what extent gastritis accounts for achlorhydria of long duration preceding carcinoma of the stomach is uncertain, and a reduction of acid cells cannot be the explanation for a majority of such cases.

SUMMARY

The incidence, severity, and distribution of the mucosal changes of chronic gastritis were determined in 199 stomachs resected for carcinoma. Employing rolls of mucosa, the entire available portions of both lesser and greater curvatures were examined in each case. Few specimens were completely free of change. Gastritic changes followed the same general patterns of distribution found in noncancerous stomachs. The antrum and body were affected independently, and in either area the changes ranged from focal to diffuse in distribution. Gastritis involved the region of the lesser curvature without comparable changes elsewhere, but extensive changes along the greater curvature were indicative of a diffuse process throughout. The body mucosa showed diffuse changes over but part of its extent in some instances. Using the findings on the greater curvature as an indicator, diffuse gastritis was found in the antrum in about 45 percent and in the body in about 55 percent of the specimens in which those areas were available for examination. Diffuse changes

ranged from mild to severe in intensity and were severe in the majority of instances. Gastritis of all degrees of severity and extent was quite independent of the size and location of the tumor. Diffuse changes in the body mucosa were constant with the few polypoid carcinomas, and the majority of normal body mucosas were found in association with infiltrative carcinomas.

Most cases which showed achlorhydria to histamine had diffuse gastritis involving the body mucosa, but only cases with severe diffuse gastritis constantly showed achlorhydria. These constituted about half of the cases of achlorhydria and a third of the group in which acids were determined.

It is not certain that diffuse gastritis precedes carcinoma in all instances where the association exists. Gastritis does not necessarily accompany carcinoma, and it is possible that mucosal alterations may develop in the presence of a carcinoma. However, even if diffuse gastritis when present always precedes carcinoma, the association obtains only in about half the cases. This proportion is not impressive and does not provide a strong support for a causal relationship between diffuse gastritis and cancer.

CONCLUSIONS

In cancer-bearing stomachs, chronic gastritis shows the same variations in distribution seen in noncancerous stomachs and may be focal to diffuse in either antrum or body independently or may involve both antrum and body to the same or in varying degree.

Diffuse gastritis in either antrum or body bears no particular relationship to the size or location of the tumor.

In the series of cases reported here only the few polypoid carcinomas consistently showed diffuse gastritis in the body mucosa.

Diffuse gastritis involved either antrum or body in only about half of the cases of carcinoma, and the proportion of these cases in which the gastritis preceded the carcinoma is uncertain.

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Chairman Palmer: Thank you, Dr. Hebbel. The third paper, Chronic Atrophic Gastritis: Identification of Different Varieties, will be presented by Dr. Alvin J. Cox.

CHRONIC ATROPHIC GASTRITIS: IDENTIFICATION OF DIFFERENT VARIETIES [1]

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Since almost nothing is known of the pathogenesis of chronic atrophic gastritis, and particularly in view of the frequent assertion of a relationship between this condition and gastric carcinoma, it seems wise to examine carefully any evidence which might provide clues to the nature of this obscure alteration in mucosal structure.

Carcinoma frequently distorts the stomach, and it may add confusing appearances to an associated gastritis, so it is probable that a better study of the nature of chronic gastritis can be made in noncancerous stomachs. The quantitative data to be presented here have been obtained from 201 carefully studied stomachs in which carcinoma was not present. I wish to emphasize some evidence which suggests that there is more than one fundamental type of chronic atrophic gastritis.

It is widely recognized that the lesions of atrophic gastritis have a different distribution in different stomachs. Antral gastritis is a term commonly used to indicate a localization of the process in the antrum or pyloric zone of the mucosa. In other cases the lesions are situated predominantly in the body of the stomach which is lined by the acid-secreting (fundic) zone of mucosa. If the lesions in these two principal zones are manifestations of the same disease process, they should be expected to occur together much more frequently than chance would dictate. Indeed, the designation pangastritis suggests that such a relation is presumed to exist.

While the incidence of all forms of chronic atrophic gastritis increases with advancing age, it has been known for a long time that the gastric lesion in patients with pernicious anemia is unusually severe, and various writers have emphasized the specific localization of this lesion in the body (fundic zone) of the stomach. It has been pointed out that in some cases the antrum (pyloric zone) is free from similar changes, the transition between the mucosal zones being abrupt. Polyp formation has been seen with unusual frequency in these stomachs. The lesions of the more common variety of atrophic gastritis, on the other hand, are often principally or entirely confined to the pyloric zone and are usually focal, without complete replacement of the preexisting mucosal structure.

Two hundred and one stomachs, obtained at autopsy before extensive post mortem changes had occurred, have been studied with respect to the relationship between corresponding changes in the 2 principal mucosal

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 14, 1948.

zones, which will be referred to as the pyloric zone and the fundic zone. The mucosa was stretched flat before fixation, and a histological study was made of 3 representative mucosal strips which extended the entire length of each stomach along the anterior wall, the posterior wall, and the lesser curvature respectively.

Two types of mucosal change have been used as criteria in the identification of chronic atrophic gastritis, infiltration of the mucosal stroma by lymphoid cells, and metaplasia of the epithelium to form intestinal-type glands. Because it was noted that the incidence of these two types of change is not the same, they have been considered separately. Although there were striking isolated exceptions, in general the degree of cellular infiltration in one mucosal zone was similar to that in the other. The fundic zone, however, was usually less heavily infiltrated by cells. This relationship between the zones was distinct and is consistent with the view that the same mechanism may act to produce the infiltration in both regions.

Examination of the occurrence of mucosal metaplasia to intestinal-type glands, however, disclosed an entirely different relationship.

The stomachs were classified according to the occurrence of metaplasia in each mucosal zone. The process was designated mild when a few isolated foci were seen, and severe when approximately one-third or more of the mucosa was altered. The moderate degrees of severity lay between these extremes. No case was included in any group unless the changes were distinct and involved the anterior or posterior wall. A few cases in which slight changes were not clear-cut, as well as several which showed only a few foci of metaplasia on the lesser curvature, were placed with the group in which no metaplasia was seen. On the whole, there was good agreement in the amount of metaplasia in corresponding parts of the different mucosal strips from the same stomach, although the change was nearly always most extensive on the lesser curvature. The strips from the anterior wall and the posterior wall showed remarkable similarity in extent and distribution of lesions in nearly every case. In a few instances there was difficulty in determining the location of the transition from pyloric zone to fundic zone, and in several cases of extensive metaplasia in the pyloric zone there were scattered foci of altered mucosa extending 1 or 2 cm. into the fundic zone. If the foci extended no more than 2 cm. above the point of mucosal transition and if the remainder of the fundic zone mucosa was free from metaplasia, such cases were classified with others in which metaplasia was limited to the pyloric zone. This limited extension of the pyloric zone lesion a short distance into the fundic zone appeared to be a condition clearly different from that in which lesions were spread throughout the fundic zone.

Table 1 illustrates the relative frequency of occurrence of epithelial metaplasia in the pyloric and fundic zones of the stomach mucosa.

TABLE 1.—*Comparison of the incidence of epithelial metaplasia in the 2 principal mucosal zones of 201 stomachs*

Incidence	Cases	Metaplasia	
		Fundic zone	Pyloric zone
Pyloric-zone metaplasia:	*Number*	*Percent*	*Percent*
None	142	14	----------
Mild	32	19	----------
Moderate or severe	27	22	----------
Fundic-zone metaplasia:			
None	169	----------	30
Mild	7	----------	43
Moderate or severe	25	----------	36

Of the 142 cases where no metaplasia was found in the pyloric zone, 14 percent had fundic zone lesions of this type, and the incidence of the same change was only 19 percent and 22 percent in cases where the pyloric zone showed mild or severe metaplasia. Similarly, the cases in which metaplasia of the fundic zone was present showed associated pyloric zone metaplasia in only 43 percent of 7 cases and 36 percent of 25 cases, while comparable pyloric zone changes occurred in 30 percent of 169 stomachs in which the fundic zone was free from metaplasia. These differences represent essentially the same incidence of metaplasia in either of the mucosal zones whether or not there was metaplasia in the opposite zone. The lack of any significant difference is made even more plain by consideration of the fact that each group of cases presenting mucosal metaplasia had a higher average age than those with unaffected stomachs, so the expected incidence of metaplasia in either mucosal zone would be higher in those instances in which the opposite zone was affected. The severity of the lesions in either zone was not recognizably different in stomachs where the opposite zone was also involved.

It is evident, therefore, that metaplasia developed concurrently in the two principal mucosal zones of the same stomach no more frequently than can be explained by chance. This is strong evidence in favor of the presence of independent etiological factors in the formation of these lesions. Since the degree of cellular infiltration of the mucosa does not show a comparable difference related to the mucosal zones, and since it does not regularly reflect the presence or the extent of metaplasia, it is possible that it is controlled by still different factors.

In view of the conflict of views concerning the relationship of chronic atrophic gastritis to gastric carcinoma, and because of the evidence that the former is not a single entity, the possible relationship should be investigated further, giving attention to possible differences in the type of gastritis which is found in association with gastric carcinoma.

SUMMARY AND CONCLUSION

The frequency of coincidental epithelial metaplasia in both of the principal mucosal zones of the stomach is in no way what would be expected if the lesions in the two regions represented results of the same

disease process. It seems probable that at least two different mechanisms contribute to the development of the changes which have been named chronic atrophic gastritis. It is possible that some of the differences of opinion regarding the relationship of chronic gastritis to carcinoma of the stomach may be resolved by further knowledge of differences in types of chronic gastritis

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Chairman Palmer: Thank you very much, Dr. Cox. I think we can well pause for a few moments for some discussion of these three interesting papers. I am reminded, in connection with the subject of the possible relationship between chronic gastritis and cancer, that Konjetzny's view is that the gastritis per se is not of significance with respect to cancer but rather the fact that as a result of the gastritis there comes about in the mucosa a continued regeneration which, in the course of time, gets out of hand and becomes neoplastic. I don't know just how one is to study that regenerative process statistically.

I think I will call on Dr. Schindler to open the discussion of this interesting group of papers, and may I request the discussants to please speak loudly, because the acoustics are not quite as good as they might be.

Dr. Schindler: I would like to summarize briefly facts concerning the relationship between atrophic gastritis and gastric carcinoma:

(1) Dr. Warren and Dr. Meissner found that the epithelial changes of atrophic gastritis correspond with those precancerous changes in other organs.

(2) In one disease, pernicious anemia, which almost always is associated with atrophic gastritis, gastric carcinoma is more frequent than in other adults of the same age group, as proved by Dr. Rigler and Dr. Kaplan.

(3) It seems, from the studies of Dr. Comfort and his coworkers, and from the presentation of Dr. State yesterday, that patients with achlorhydria develop more gastric cancers than other adults of the same age group. But what is the significance of achlorhydria? We know from gastroscopic studies that it rarely occurs together with an undoubtedly normal mucosa. I found only two percent, although Dr. Carey's percentage is higher. Achlorhydria is usually a sign of the disease entity of superficial atrophic gastritis. Also, Dr. Comfort suggests that achlorhydria is often the result of atrophic gastritis.

(4) Two statistics, those of Kapp and of Usland, have shown that patients suffering from an organic gastric disease other than ulcer, most likely gastritis, develop gastric carcinoma in 13 percent of the cases.

(5) Benign adenomas develop seven times more frequently on the soil of atrophic gastritis than in a normal mucosa, as proved by gastroscopic statistics. Today Dr. Comfort showed that achlorhydria often precedes by years the development of benign adenomas.

(6) In an increasing number of cases, direct proof is possible that atrophic gastritis preceded the development of gastric carcinoma. It cannot be admitted that atrophic gastritis is an unspecific response to gastric lesions, as suggested by Guiss and Stewart. Only rarely does one find in gastric ulcer that type of extensive atrophic gastritis which is so common in gastric carcinoma.

In gastroscopic observation there is all the difference in the world between the aspect of the stomach of the healthy aged adult, that of the average ulcer stomach, and that of the average carcinoma stomach. We are trying in Los Angeles to do for atrophic gastritis what State and Comfort did for achlorhydria. It will take time, however. In the meantime the research on etiology of atrophic gastritis must go on. The significance of deficiency states is known, but other factors may be important also.

Dr. Hollander showed yesterday the origin of cysts after destruction of the mucus barrier, and I think that his was a most valuable contribution.

Chairman Palmer: I am told that Dr. Guiss is in the audience. We are all familiar with his and Dr. Stewart's classic contribution on the subject, and since there is

a different point of view between that presented by Dr. Schindler and the essayist, I think we will all be glad to hear from Dr. Guiss on this subject.

Dr. Guiss: I have been very interested in the papers this morning because they followed so closely the subject of Dr. Stewart's and my own work. We have continued with our studies, and now that more material is available of the proper type for study we have made an analysis somewhat similar to Dr. Hebbel's of the distribution of gastritis in association with various types of gastric lesions.

Using ulcers as our first group, we went over suitable specimens of stomachs that were removed for duodenal ulcer. We found, in cases of duodenal ulcer, that the stomach was either devoid of gastritis, so-called chronic atrophic gastritis, or it was sharply limited to the pylorus itself, and one might very easily assume that the gastritis present was merely a spread of the inflammatory reaction and damage in the duodenum itself.

When the peptic ulcer was located in the pyloric portion of the stomach, again we found that the gastritis was limited rather sharply to the immediate neighborhood of the lesion itself. It is quite common to find that the fundus is completely devoid of the features of chronic atrophic gastritis. However, when the peptic ulcer was located in the fundus, we found almost invariably a pangastritis, usually rather severe in degree.

Passing on, then, to the specimens containing gastric carcinoma, we found that without respect to the size of the lesion or, in most cases, its location, in general there tended to be a chronic atrophic gastritis of severe degree, and it was usually rather generally distributed over the entire gastric mucosa. In other words, even though the lesion was in the pylorus, the gastritis tended to involve the fundic areas too, and the converse was true.

Of course, we are speaking of large numbers now, and individual exceptions to these statements are frequent, and I know we all have run across them. One interesting thing we found in regard to distribution of gastritis in gastric carcinoma was that when the lesion was confined to the cardiac region, the gastritis seemed to be much more inclined to be limited to that area alone; and it was not uncommon to find the gastric mucosa of the pylorus and fundus almost normal. What significance this has we are unable to state. All our figures have been very carefully tested statistically, and there is a very definite diminution in the distribution and the degree of gastritis when the cardiac region alone is involved by cancer.

In addition, we were interested in the problem of anacidity, particularly with reference to chronic atrophic gastritis. We ran, as carefully as we could, an assay of the number of normal-appearing parietal cells present in the mucosa and correlated this with the hydrochloric acid figures that were obtainable. I must confess the correlation was far from perfect. However, statistically tested, we found that there was a very high positive correlation between the number of parietal cells present in the stomach and the free hydrochloric acid figure obtained on histamine test, and also there was, as you would expect, a rather high negative correlation between the degree and extent of the chronic atrophic gastritis present and the number of parietal cells.

It is true that you will occasionally find gastric mucosas in which there is apparently completely normal fundus mucosa with beautifully preserved parietal cells, and still you will have a histamine-fast achlorhydria. This we cannot explain, but it is rather uncommon. We felt rather strongly, speaking in general, that the degree of chronic atrophic gastritis would usually explain the diminution or absence of hydrochloric acid in the tests.

Chairman Palmer: Thank you very much, Dr. Guiss. Dr. Shay, would you like to continue the discussion?

Dr. Shay: Gastric anacidity constantly recurs in our discussions of the gastric cancer problem. Since so much emphasis is placed upon this finding, I think it might be well to re-examine the clinical methods by which we arrive at the conclusion that gastric anacidity exists in a particular patient. The usual procedure is to titrate gastric samples obtained after a test meal using Toepfer's reagent as indicator in titrating for free hydrochloric acid and phenolphthalein for total acidity. When the

addition of the Toepfer's reagent to the gastric sample yields a yellow color, the gastric sample is considered to have no free hydrochloric acid, and if all the gastric samples react in the same way, the patient is considered to have gastric anacidity, and a true anacidity if the same results are obtained under an adequate dose of histamine.

I agree completely with the remarks made by Dr. Dunlap yesterday, that when dealing with anacidity in an attempt to evaluate the degree of impaired parietal cell activity, the use of indicators alone will not suffice. The appearance of a yellow color after the addition of Toepfer's reagent to all the samples obtained in a fractional gastric analysis is only an indication that none of the extractions have a pH below 3.5. However, the range of pH represented by those samples may run from pH 3.6 to 8.5. If one measures the pH of these so-called anacid samples, one may find a wide variation of pH even though all appear to be anacid by titration alone. If one considers that a drop of only one pH unit means a tenfold increase of hydrogen ions and a drop of 2 pH units a hundredfold increase, the desirability of measuring the pH of the gastric samples and not depending upon titration alone becomes obvious. This is especially important after the use of histamine. Under such circumstances one frequently finds that parietal cells that would be classed as having failed to respond to histamine stimulation when the acidity of the gastric samples is measured by titration must be considered as still able to secrete acid when pH changes of the various samples are determined.

While the above considerations in no way alter the importance of gastric anacidity as it relates to the gastric cancer problem, pH determinations of the gastric samples in anacidity certainly yield a clearer picture of the activity of gastric mucosa.

Chairman Palmer: Thank you, Dr. Shay. I am sure we would be very glad to have general discussion of these papers now and questions directed to the essayists, whichever one you may have in mind. Do we have further discussion?

Dr. Rigler: I would like to ask Dr. Comfort one question. I was very much interested in the difference between carcinomas and benign tumors so far as the presence of free acid is concerned, particularly in relationship to the size of the lesion. I am wondering whether carcinomas of small size in which there was a fairly respectable amount of free hydrochloric acid were not the local ulcerating carcinomas or perhaps very local infiltrating carcinomas. On the other hand, I suspect that in the so-called polypoid type of carcinoma which often looks grossly so much like a benign adenomatous polyp, even when the lesion is small, there would be an anacidity.

Did you classify your cases in that fashion to give us an idea as to this point? Obviously, when an infiltrating carcinoma becomes large enough, or even when an ulcerated lesion becomes large enough, an anacidity may also occur.

What I am trying to convey is that there may be two kinds of lesions in the stomach, one of which is closely related to the benign polypi and, therefore, would have anacidity prior to the development of the lesion. The other might not have any relationship to the background of anacidity, such as is seen in polypoid lesions; therefore, it might begin in a stomach in which the acids were normal. If such a lesion became large enough and extensive enough, anacidity might supervene.

Chairman Palmer: Thank you, Dr. Rigler. Is there any further discussion? Dr. Ross Golden.

Dr. Golden: I would like to ask both Dr. Hebbel and Dr. Cox whether their studies included the whole wall of the stomach or only the mucus membrane. On X-ray examination we often see serious disturbances in the motor function of the antrum which, in some cases at least, are associated with submucosal edema. This edema may or may not, but probably does, play a part in this motor disturbance. This is ordinarily termed antral gastritis with spasm and is often associated with hypertrophy of the pyloric and prepyloric muscle. Many years ago, Konjetzny made the statement that hypertrophy of the pyloric muscle was invariably associated with, or was due to, gastritis.

These cases constitute one of our most serious problems in attempting to demonstrate carcinomatous changes in the presence of inflammatory mucosal changes.

Here again we have the problem of trying to determine what spot on the film corresponds to what spot on the specimen. The stomach shrinks when the surgeon takes it out, but we do not know how much it shrinks. When it is put into a preservative, it shrinks still more. Can the surgeons help us by measuring in vivo the length of the area to be resected and then determining what the average shrinking in a series of cases amounts to?

A very good book on carcinoma of the stomach has been published by a Frenchman, Dr. René Gutmann. He points out that early cancerous changes on X-ray examination may be associated with very small abnormalities visible on the margin of the stomach shadow. The problem is to be sure that the abnormal contour is due to early carcinoma and not to inflammation alone. More accurate correlation of shadows on the film with the surgical specimen is necessary.

Chairman Palmer: Thank you, Dr. Golden. Dr. Althausen of San Francisco.

Dr. Althausen: I would like to ask Dr. Cox whether he tried to correlate the size and weight of the stomach with the nutritional state and especially any loss of weight on the part of the patients. The reason I am asking this question is that we have conducted some studies of the total area of the intestinal mucosa, depending on the loss of weight in rats. This is a rather fundamental question in nutritional experiments when animals lose certain amounts of weight, whether we are to assess diminutions in absorption according to diminution of weight or the area of the intestinal mucosa. We found when we quantitatively reduced the nutrition of the rat that, up to loss of weight of 25 percent, the intestinal mucosa does not increase in area, but losses of weight beyond that resulted in the reduction of the total surface of the intestinal mucosa. I thought perhaps changes in the total area of the gastric mucosa and the weight of the stomach might be a secondary phenomenon, perhaps due to the more severe effects of the gastritis in the pyloric area rather than in the antrum.

Chairman Palmer: Thank you, Dr. Althausen. Is there further discussion? Dr. Templeton.

Dr. Templeton: I would like to add to the discussion of Dr. Rigler and Dr. Golden.

It would be helpful to us as radiologists if the gastric neoplasms were classified under the great categories of polypoid, ulcerating and infiltrating.

I have been very much impressed with the early lesions found by Dr. Rigler and others. These lesions are primarily the polypoid or ulcerating types. This must be so by the very nature of the growth of the lesion.

A lesion which extends into the lumen as does a polypoid mass or which protrudes from the lumen as does an ulcer is much easier seen than is the flat lesion that extends sheet-like through the wall. These flat lesions are particularly hard to identify when they lie high in the stomach above the site of origin of peristaltic waves.

The infiltrating lesions are sometimes small and assumed to be in an early stage from the point of size. We are disappointed when the surgeon finds widespread metastases from these lesions.

Dr. Palmer, 2 years ago, indicated that these particular lesions are the great killers, and I think, Dr. Palmer, I am quoting you correctly when I say there are no authenticated cures in this particular type of carcinoma.

In reference to Dr. Golden's discussion on the antral changes, I think that there is a relationship between lesions occurring higher in the stomach and changes found in the antrum.

This relationship used to be passed off as spasm of the antrum because the antrum is narrow. I have always looked upon spasm as a very poor term. The term is confusing and muddles my thinking. It is easily used for a washbasket.

I don't think the narrowed antra are of spastic origin. They often persist with little change for long periods of time after an ulcer heals.

I think most radiologists look upon these narrow antra as a sign of something wrong elsewhere in the stomach.

When a narrowed antrum is encountered we do not put our whole attention on the antrum but search the rest of the stomach carefully. Frequently either a benign ulcer or a cancer situated higher in the stomach is found.

A number of years ago, Dr. Schindler and I studied these particular lesions. Sometimes he reported them normal, sometimes he reported gastritic changes of one type or another.

The thing that impressed me, however, was that in a number of instances he found ulcerations high in the stomach which we could not find at radiologic examination. They were too shallow.

Chairman Palmer: Thank you, Dr. Templeton. Is there further discussion?

Dr. Schiff: A number of years ago my associates and I studied the anatomical changes in stomachs obtained from patients with gastric carcinoma and patients with gastric duodenal ulcer and attempted, at that time, to correlate the anatomical changes with the gastroscopic changes.

In comparing the anatomical changes found in our patients with ulcers, particularly gastric ulcers, with those seen in patients with gastric cancer, we were very much impressed with the similarity in the character of the findings, e. g., the frequency of cystic dilatation and goblet-cell metaplasia. This led us to question the specificity of gastritis as a precursor of either cancer or ulcer. I would like to ask Dr. Guiss if he has noticed any difference in the quality of the anatomical changes in the patients with ulcer as compared to the patients with cancer?

Chairman Palmer: Dr. Guiss, would you answer that question now?

Dr. Guiss: We found that if the peptic ulcer was located in the fundus of the stomach, the degree and extent of the gastritis, measured on a statistical scale, was identical with that found in gastric carcinoma. It is only when the ulcer is located in the pyloric region that you find the changes limited to the pylorus. The changes in the fundus in these cases are usually quite limited. When the ulcer is in the fundic area, the gastritis, both in extent and degree, is the same as seen in gastric carcinoma.

Chairman Palmer: Thank you, Dr. Guiss and Dr. Schiff. Is there anything further? Dr. Hollander.

Dr. Hollander: Dr. Rigler has raised a question which I think merits very serious consideration this morning. It is the question of when is an anacidity not an anacidity. For one thing, I have had repeated experience, and I think most of you have also, of performing a histamine test on a patient one day and getting a clearly negative response, but on repeating the test 1 or 2 weeks thereafter, getting a report of positive secretory action. Another aspect of the problem is the one Dr. Shay, himself, has reported. He commented on a fact sometimes observed when you work with a patient in whom there is no obvious free acid by the usual criteria. In spite of this, you find that following the injection of histamine, the pH falls from a value of 7.0 or a little bit higher, down to near 3.5, which is the free acidity end point which we generally accept, but it never reaches this boundary value. Thus, there is a progressive change in pH in the direction of a free acid value, in spite of the fact that an obvious free acidity value is never attained. This suggests that the parietal cells are functioning to some slight extent but that the small amount of acid is being neutralized by mucus or other nonacid component of the stomach secretions.

We have encountered similar instances in our work with insulin-hypoglycemia in the study of ulcer patients following vagotomy. We find, not infrequently, that such a patient will give no free acid following the injection of an adequate dose of insulin, but that there is a marked rise in the combined acidity curve. Although we label such a case negative so far as response to insulin hypoglycemia is concerned, the results suggest that there may be a small amount of acid secreted in spite of a complete vagotomy, a small amount which is being neutralized and, therefore, yields a false anacidity.

I think such a differentiation as Dr. Shay proposes must be taken into consideration in our analysis of the anacidity problem. Whether the phenomenon is significant clinically is another matter.

Chairman Palmer: Thank you, Dr. Hollander. Is there further discussion?

Dr. Bloomfield: May I say a word? I agree with both Dr. Shay and Dr. Hollander about the fact that there is a group of patients in whom the pH of gastric secretion falls

a little bit from seven or eight but not enough to show free hydrochloric acid with the ordinary test. We went to a lot of work over a long period of time measuring pH's in all such people, but then it turned out that such a reaction happens uncommonly compared to a lack of change. As a rule there is a true neutrality, and it is only once in a very long time that one gets this minute change, so it is questionable whether it is worth while bothering about that in practice.

The other thing I find interesting in all of this discussion of gastric secretion is that no one has had much to say about volume, which is just as important as acid, because what one wants to know is the total rate of acid secretion, not concentration. This has been pointed out thoroughly, I think, in connection with other body fluids, with urine, for example, and it makes a great deal of difference whether such a change of pH occurs in a patient pouring out a large amount of fluid or whether it occurs in a patient with little gastric juice.

I think it is a rather barren discussion. There must be some point between a total destruction of acid-secreting cells and a few remaining cells which would be of little practical importance and yet might account for these patients who lower their pH a little bit but who have no measurable amount of gastric secretion that way. It must be a matter of a few remaining cells and not a matter of some fundamentally different pathological process in the stomach. I think the study of all gastric secretion should include a test in which volume is measured as well as acidity.

Chairman Palmer: Thank you very much, Dr. Bloomfield. Dr. Shay has a remark to make.

Dr. Shay: May I say a word in answer to Dr. Bloomfield's discussion? I am afraid that I must differ with the opinion expressed by Dr. Bloomfield in that I believe it is important to insist upon exact definitions of terms in clinical medicine just as we do in the basic sciences even though in their application a certain elasticity of meaning may be indulged in. I can not admit that a loose terminology can be useful even in clinical medicine. Such a loose terminology I am convinced is brought about when discussions of gastric anacidity are based on results obtained by titration of gastric samples alone.

Whether or not small changes in pH in gastric samples are significant clinically does not to my mind alter the fundamental considerations of the problem. It is my belief that we are all classifying many patients under gastric anacidity and even as failing to respond to histamine, when actually they represent instances of low rates of parietal cell secretion into a gastric lumen where the buffering mechanism is such as to maintain the gastric contents at a pH above 3.5. This, however, does not justify the use of the term anacidity (without acid) and would not be used if the degree of change in pH that occurs following the test meal or the use of histamine were known. From a practical standpoint, I believe it is important to take cognizance of the above, since the clinician in some diseases such as pernicious anemia, for example, places so much importance on the gastric secretory response to histamine.

Dr. Bloomfield: I have to say one word more. Of course, titration under those conditions doesn't have any meaning, and to really find out what is going on in the stomach, one has to measure total base and total chloride. We have done that; it is all in the anacidity book. There is a lot of base there in terms of concentration, but the total amounts are so small, if you take anything like normal gastric juice, they become negligible and insignificant.

Another thing is that all of these people with anacidity have not had anacidity all their lives. We know that in their twenties there are perhaps 10 percent of the people with anacidity, and at 60 there are 40 percent. Somewhere along the line they lose their gastric juice. I can't see that it makes any difference whether the patient has reached the final end point or whether he has dying cells in his stomach giving their last gasp and which in a few years won't be secreting anything at all. It seems to me to be a rather artificial distinction.

Chairman Palmer: Thank you, Dr. Bloomfield. This is a rather interesting postscript to the anacidity book which we must all read. Dr. Meissner.

Dr. Meissner: Dr. Schiff has raised an interesting question as far as chronic gastritis

being common. The fact that chronic gastritis is common doesn't necessarily mean that it is a precancerous lesion. Conversely, that chronic gastritis is common doesn't mean it is not a precancerous lesion. It doesn't prove anything either way. The important thing is the degree of chronic gastritis and whether there is a type of chronic gastritis which is comparable to precancerous lesions in other parts of the body. A few years ago Dr. Warren and I did a study on a series of stomachs, and we found in a few cases of chronic gastritis epithelial changes which were quite comparable to generally accepted precancerous lesions in other structures of the body. We also found, in comparing stomachs resected for cancer with stomachs resected for peptic ulcer, that the degree of this change is greater in the stomachs that have cancer.

Chairman Palmer: Are there any other questions?

Dr. Templeton: I would like to have Dr. Comfort answer Dr. Rigler's question.

Chairman Palmer: If there are no further questions, we will give the essayist an opportunity to get into the discussion. Dr. Comfort, will you please answer Dr. Rigler's question?

Dr. Comfort: Before answering Dr. Rigler's question may I make the point which today's discussion appears to emphasize; namely, that a close correlation between the degree of atrophic gastritis and the secretory activity of the stomach, as measured today, is not expected. Dr. Rigler, if I understand his question correctly, has asked whether the small carcinomas, occurring as they do in stomachs whose secretory activity is characterized by a nearly normal percentage of anacidity, are the localized ulcerating and localized infiltrating lesions, and the larger lesions, occurring as they do in stomachs whose secretory activity is characterized by a high percentage of anacidity are the polypoid types. Dr. Rigler also suggests that the small lesion associated with anacidity may be different from the small lesion associated with free acid. My associates and I have not attempted to correlate the gross type of lesion with the secretory activity of the stomach; it is merely an impression that the large majority of the small lesions were ulcerative and few were localized infiltrating and polypoid in type.

Chairman Palmer: Is your question answered, Dr. Rigler?

Dr. Rigler: Yes.

Chairman Palmer: Dr. Hebbel.

Dr. Hebbel: Just a word or two in answer to Dr. Golden's question relative to edema. In my experience submucosal edema is very common in association with gastric cancer and may involve all of the resected segment beyond the tumor.

In regard to the discussion between Dr. Rigler and Dr. Comfort, I should like to add that in our material we have no examples of either polyp or polypoid carcinoma in the absence of extensive atrophy.

Chairman Palmer: Thank you very much. Dr. Cox.

Dr. Cox: One of the things which has struck us in examining these stomachs with severe chronic atrophic gastritis is that the evidence of reaction in the submucosa and deeper tissues is minimal. Whereas destructive processes like obvious ulcers produce fibrosis and considerable adhesion between the mucosa and the underlying submucosa, in stomachs with most severe gastritis there may be no greater anchoring of the mucosa to the underlying structures than in normal stomachs,

To answer Dr. Althausen regarding the relationship of nutrition to the size of the stomach, we examined that carefully and were unable to detect any relationship. Obese people had the same range in stomach size as others, and, interestingly enough, the size of the individual bore little relationship to the size of the stomach. There was approximately threefold variation in area from case to case, and it was quite unrelated to variations in body size or almost anything else that we could find except sex.

Chairman Palmer: Thank you, Dr. Cox. There are two remaining papers on the program before the general discussion. The first is by Dr. William A. Meissner on Malignancy of Gastric Cancer.

MALIGNANCY OF GASTRIC CANCER [1] [2]

By WILLIAM A. MEISSNER, M. D., *Laboratories of Pathology, New England Deaconess Hospital, and Harvard Cancer Commission, Boston, Mass.*

One characteristic of malignant tumors is their ability to metastasize to regional lymph nodes. The rapidity and extent to which such lymph-node metastases occur are usually among the more reliable criteria on which one can judge how highly malignant is any individual cancer. Extent of metastases to regional lymph nodes has been used rather extensively as a prognostic index for carcinomas of the intestine and breast and recently has been advocated for carcinoma of the stomach by Moore et al. (*1*).

The low cure rate of gastric cancer is well known. Yet we have been impressed by the seemingly large number of resected specimens which have had little or no demonstrable tumor in the regional lymph nodes. This suggested that a survey of the lymph-node involvement in resected specimens might be of interest; first, to determine whether there is any correlation of presence or absence of metastases with certain clinical, gross, and microscopic features of the primary cancer, and, second, to determine if, contrary to popular belief, many gastric cancers are being resected early as far as lymph-node metastases are concerned, but that other characteristics of malignant growth are equally important in determining the final outcome.

With these questions in mind, a study of the lymph-node involvement in 100 resected specimens of carcinoma of the stomach was undertaken. Practically all of the specimens were resected during the last 5 years, so 5-year-cure rates are not available. They were chosen at random, except that to insure a reasonably adequate examination, no specimen was used unless a minimum of 15 lymph nodes could be isolated. In the majority many more were found, and all nodes were examined microscopically. In addition to the lymph-node study, the primary tumors were graded microscopically; their location, size, and gross appearance were tabulated, and each case was carefully examined for the presence or absence of blood-vessel invasion by the tumor. Verhoeff elastic tissue stain with van Giesen counterstain was used frequently to assist in the latter determination.

In order to facilitate comparisons, the cases were divided into three groups (see tabulation): No metastases in lymph nodes (28 cases); metastases in less than half the lymph nodes (44 cases); metastases in half or more of the lymph nodes (28 cases).

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[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 14, 1948.

[2] Aided in part by grants from the American Cancer Society, Massachusetts Division, Inc., and the Anna Fuller Fund.

INCIDENCE OF LYMPH-NODE METASTASES

Nodes involved:	Number of cases
None	28
Less than half	44
Half or more	28
Total	100

Of the 72 males and 28 females there was no apparent difference in lymph-node metastases. Neither was there any obvious correlation with age, although of the 4 tumors in the 30–40 year group, none showed metastases to more than half the nodes. Neither the portion of stomach nor the curvature or wall on which the primary lesion was situated had any apparent relationship to the presence or extent of lymph-node metastases.

Table 1 shows size (measured as greatest diameter) compared with lymph-node metastases. Here again there is little correlation, metastases frequently being present in small tumors and at times being absent in large tumors. No tumor over 12 cm. was without lymph-node involvement, however. Four cases were so diffuse that exact gross measurement was not reliable.

Table 2 shows lymph-node involvement correlated with Borrmann's classification. The determination of Borrmann type from gross description is not entirely satisfactory but was facilitated in this series by the availability of Kodachrome photographs on many of the specimens and because many cases were originally described with the Borrmann typing in mind. As might be expected, Borrmann types I and II showed relatively less node involvement and the types III and IV relatively more, but this was inconstant.

In table 3 the primary tumors have been classified into three groups according to microscopic malignancy, using differentiation as the most important criterion. Such microscopic classification was not always satisfactory since a variability (Pl. 78, fig. 1) was frequently found within both the primary tumor and within the metastases.

TABLE 1.—*Size of primary tumor*

Lymph-node involvement	Size				
	0–3 cm.	3–6 cm.	6–9 cm.	9–12 cm.	12 + cm.
None	3	5	12	7	0
Less than half	3	17	13	8	1
Half or more	1	7	12	3	4

TABLE 2.—*Gross type of tumor*

Lymph-node involvement	Borrmann I and II	Borrmann III and IV
None	16	12
Less than half	17	27
Half or more	1	27

TABLE 3.—*Miscroscopic type of tumor*

Lymph-node involvement	Malignant adenoma (well-formed glands)	Adeno-carcinoma (poorly-formed glands)	Carcinoma simplex (diffuse growth)
None	7	10	11
Less than half	4	10	30
Half or more	1	4	23

The results of the survey as far as presence or absence of lymph-node metastases are comparable to the findings of Coller et al.(*2*) who similarly examined the distribution and extent of lymph-node metastases from gastric carcinoma in 53 cases. There is little or no relationship of presence or extent of lymph-node metastases with age, sex, or size and location of the primary tumor. Those tumors which were of lower Borrmann types and of the lower microscopic grades tended to have fewer metastases, but this was inconstant and did not hold for any individual case but for the group as a whole.

With such a high percentage of resected specimens having little or no lymph-node metastases, it is obvious that other factors of malignant growth are of importance since the cure rate is so low. In examining the primary tumors and in taking multiple sections of the grossly uninvolved stomach, we have been impressed with the frequency with which diffuse lymphatic spread (Pl. 78, fig. 2) takes place. The perineural lymphatics, particularly, may be involved, often with little demonstrable lymph-node involvement. The lymphatic spread may extend across the pylorus to the duodenum (Pl. 79, fig. 3), as emphasized by Castleman (*3*) and others (*2*) as well as to the proximal line of resection. We have been surprised at times to find tumor microscopically at both margins of surgical resection when the gross margins appeared quite adequate. The surgeon is obligated to remove as wide proximal and distal margins as feasible in every instance to prevent local recurrence.

Still another characteristic of malignant tumors is ability to invade blood vessels and subsequently metastasize by blood stream (Pl. 80, fig. 4). In this series blood-vessel invasion was found in 57 percent (table 4) and showed little relationship to the presence or absence of lymph-node metastases. Neither was there apparent correlation of blood vessel

invasion with age or sex, or with size, location, microscopic (table 5) or gross (table 6) appearance of the primary tumor. A study of the significance of such blood-vessel invasion and subsequent metastases is currently being done.

TABLE 4.—*Blood-vessel invasion incidence*

Lymph-node involvement	Present	Absent
None	13	16
Less than half	26	17
Half or more	18	10
Total	57	43

TABLE 5.—*Microscopic type of tumor and blood-vessel invasion*

Tumor type	Blood-vessel invasion	
	Present	Absent
Malignant adenoma	3	9
Adenocarcinoma	19	6
Carcinoma simplex	35	28

TABLE 6.—*Gross type of tumor and blood-vessel invasion*

Tumor type	Blood-vessel invasion	
	Present	Absent
Borrmann I and II	19	16
Borrmann III and IV	38	27

SUMMARY AND CONCLUSIONS

A survey of the extent of lymph-node metastases in 100 resected specimens of carcinoma of the stomach showed no lymph-node involvement in 28 percent and less than half the isolated nodes involved in another 44 percent. There was no constant relationship found between extent of lymph-node metastases and clinical, gross, or microscopic features.

Other factors than ability to metastasize to lymph nodes are of importance in determining the malignant course of gastric carcinoma. Such factors include diffuse lympathic invasion, often with little lymph-node involvement, and blood vessel invasion.

Blood-vessel invasion, present in 57 percent of the cases, showed no correlation with extent of lymph-node involvement and no constant correlation to gross or other microscopic features of the primary lesion.

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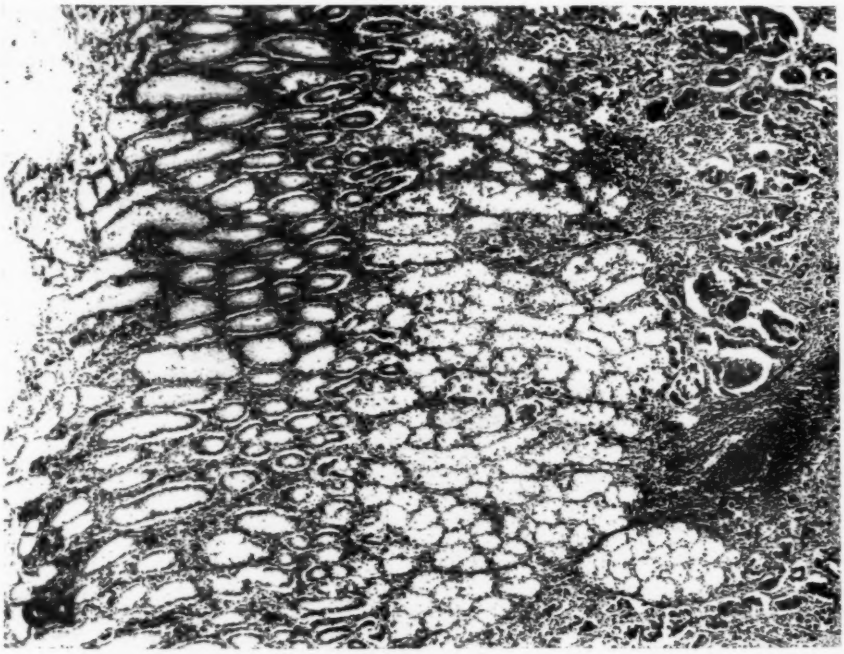
Chairman Palmer: Thank you very much, Dr. Meissner. The next paper will be by Dr. Stanley R. Friesen of Minneapolis. It is The Silent Interval in Gastric Cancer: A Study of the Time Elapsing Before Reassertion of Symptoms in Patients with Microscopic Malignancy in the Proximal Line of Excision and Without Lymph-Node Metastases.

PLATE 78

FIGURE 1.—Marked variability in microscopic appearance within a primary tumor. Such variations make microscopic classification difficult. Eosin-methylene blue. ×50.

FIGURE 2.—Diffuse spread of carcinoma in submucosal lymphatics. Overlying mucosa is not ulcerated. Eosin-methylene blue. ×50.



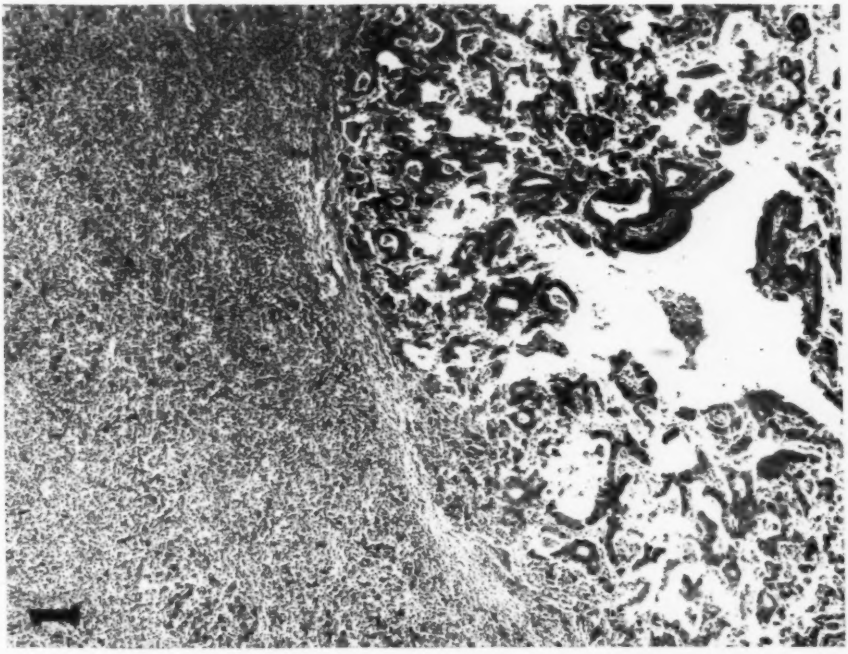


PLATE 79

FIGURE 3.—Diffuse spread of carcinoma in duodenal mucosa. Eosin-methylene blue. ×50.

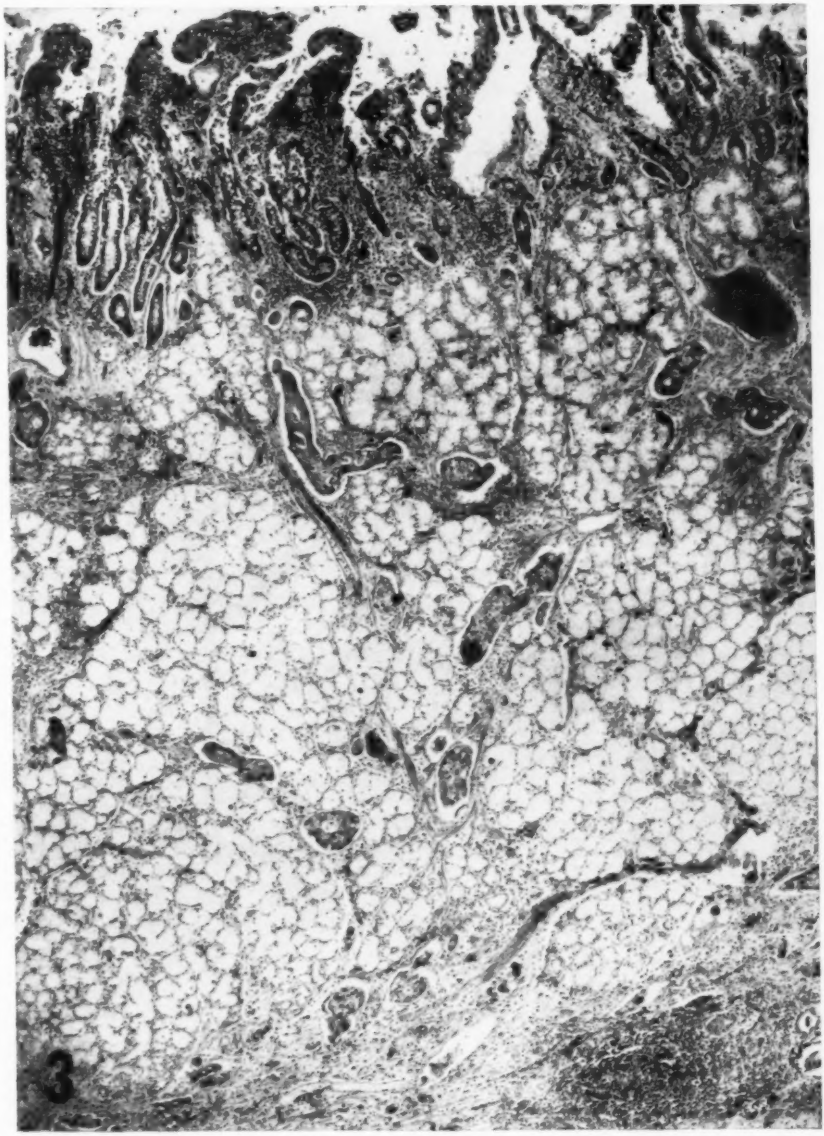
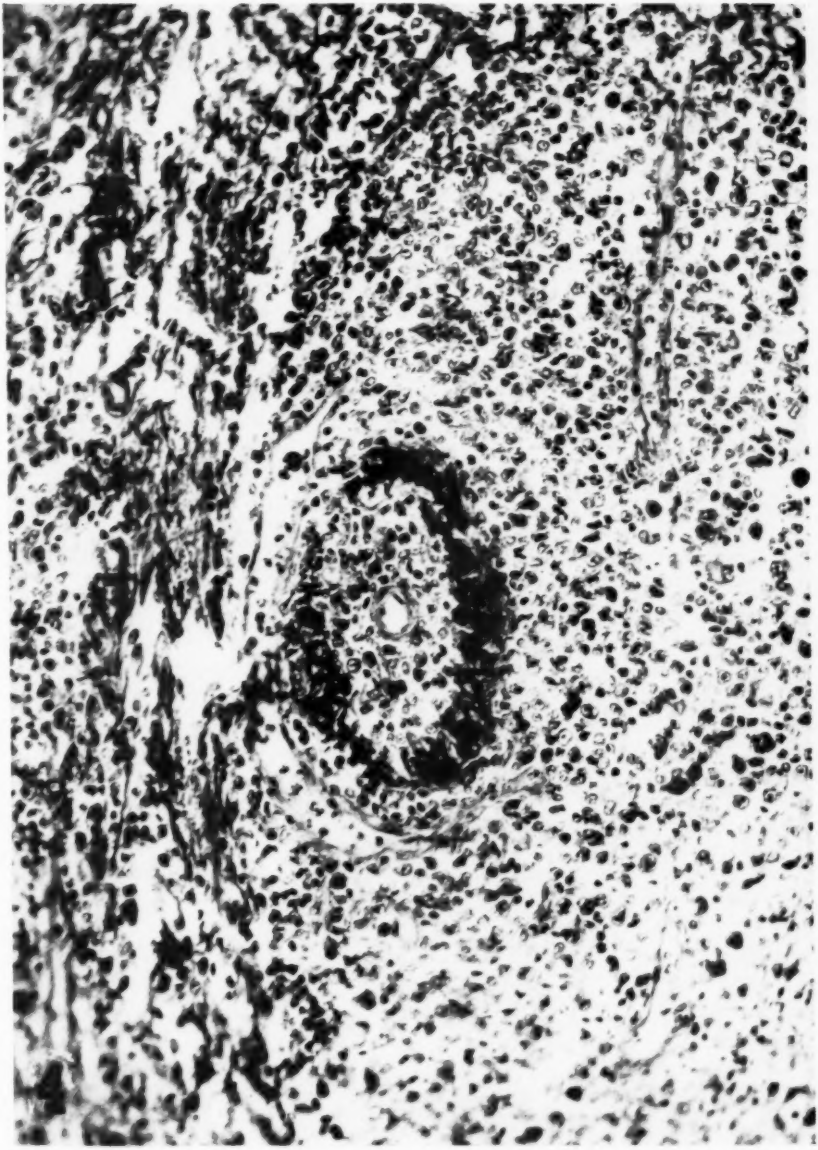

3

PLATE 80

FIGURE 4.—Tumor growing within blood vessel. Elastica of vessel is stained black. Verdhoeff, van Giesen. ×250.



THE SILENT INTERVAL IN GASTRIC CANCER: A STUDY OF THE TIME ELAPSING BEFORE REASSERTION OF SYMPTOMS IN PATIENTS WITH MICROSCOPIC MALIGNANCY IN THE PROXIMAL LINE OF EXCISION AND WITHOUT LYMPH-NODE METASTASES [1] [2]

By STANLEY R. FRIESEN, M. D., *Department of Surgery, University of Minnesota Medical School, Minneapolis* [3]

Occasionally a subtotal gastric resection for gastric carcinoma performed ostensibly as a curative measure becomes only a palliative procedure by virtue of the fact that microscopic carcinoma cells remain in the line of excision and continue to grow, silently and unnoted by either patient or surgeon. Certainly a majority of gastric resections become palliative in nature by other means, chiefly in that metastases to lymph nodes and other structures have occurred by the time of resection.

As shown by Moore, State, Hebbel, and Treloar (*1*), the greatest single factor of prognostic importance determining the percentage of survival in patients resected for gastric carcinoma is the presence or absence of lymph-node metastases at the time of resection. In a 10-year survey of the results of surgical treatment of carcinoma of the stomach by State, Moore, and Wangensteen (*2*), it is noted that the number of months of palliation, in those patients who survived gastric resection between the years 1936 and 1943 but who subsequently expired from carcinoma of the stomach, averaged approximately 22.4 months. The survival time in patients resected for gastric carcinoma in the presence of hepatic metastases averaged 15 months. It should be noted that the survival time is not synonymous with the silent interval following resection, but the difference is not great. Gray (*3*), in a study concerning the clinical and pathological factors that influence the ultimate prognosis following resection of carcinoma of the stomach, states, "the three most important factors that decrease the possibility of a long postoperative life are lymphatic involvement, serosal involvement, and a tumor of high grade malignancy." He adds, "due to the fact that the incidence of lympathic involvement increases in direct proportion to the increase in severity of the malignant process as indicated by the grade, the last named feature should be considered the most important single factor." The circumstance that the present study concerns itself with cases in which tumor cells remain in

[1] Read at the Fourth Conference on Gastric Cancer held at Stanford University School of Medicine, San Francisco, December 14, 1948.

[2] Supported by a grant from the National Cancer Institute, National Institutes of Health, Public Health Service, the Flora L Rosenblatt Fund for Cancer Research, and the Malignant Disease Research Fund of the University of Minnesota.

[3] Now in the Dept. of Surgery, University of Kansas Medical Center, Kansas City, Kansas.

the proximal line of excision indicates in itself that the lesions dealt with at the time of gastric resection were of the infiltrating variety, Borrmann types III and IV.

The manner of the direct extension of the primary carcinoma of the stomach was described in 1900 by Cuneo (*4*) in the first extensive study of the subject. He stated that in cases of gastric carcinoma the submucosa was invaded early and to a greater extent than the mucosa of the stomach. He demonstrated that extensions follow the lymphatics. Verbrugghen (*5*), in 1931, in a study of 50 resected stomachs for carcinoma, confirmed Cuneo's work, and showed that submucosal extension occurs in approximately three-fifths of cases of gastric carcinoma, mucosal extension occurring in one-fifth of the cases, and serosal extension in a little less than one-fifth of the cases. He stated that the average lateral extension in Grade III carcinomas of the stomach was 6.5 mm. while that in Grade IV lesions was 10.6 mm., but showed no limit in the maximal extension in some cases. The proximal line of resection was involved in 9 of the 50 cases studied by him. The cephalic extension of carcinoma of the gastric cardia was studied in 1947 by Brownson (*6*) who showed that with lesions situated in the cardia, the esophagus was invaded in 26 of 27 cases studied. The average amount of esophageal involvement in these cases was 8.7 mm., and microscopic extension beyond macroscopic involvement proved to be present in 3 of 27 cases for a maximum amount of 5 mm. on the prepared slide, which probably represents a microscopic extension of 15 mm. in the fresh specimen. Marvin (*7*) in 1947, on the other hand, found that intraduodenal spread of malignant lesions of the gastric pylorus and antrum occurred in 38 percent of the 100 resected specimens studied. Of the cases in which the duodenum had been invaded at the time of resection, 47.3 percent demonstrated tumor cells at the line of excision, and all such patients followed died of carcinoma within a year of the time of resection.

The malignant cells left behind in the proximal line of excision at the time of resection remain relatively unmolested and grow in the same environs that the primary tumor inhabited before subtotal resection. The continued growth of the only remaining tumor cells which lie in the proximal line of excision may mimic the early course of the disease prior to the gastric resection.

Furthermore, the interval of freedom from symptoms following such palliative gastric resections may give some indication of how long it takes a cancerous tumor which already has exhibited invasive qualities to grow and reassert itself.

Recently maximum stress in the problem of gastric cancer has been on the subject of the earlier diagnosis of this disease in the hope that more patients will thus be subject to a chance for cure by the surgeon. It is, however, difficult in many instances to define what is meant by an early diagnosis or early case. The fact that there is undoubtedly a period of time in the growth of a localized cancer of the stomach during which no symptoms are produced is evidence that gastric cancer is a silent disease.

It remains to be shown what length of time constitutes the silent period of growth, the knowledge of which may facilitate earlier treatment of the patient and certainly a better understanding of the natural course of the disease at or near its inception. To gather information on this point, it may be appropriate to study the case records of those patients in whom a subtotal gastric resection for gastric carcinoma has been performed and in whom microscopic cancerous cells have been left in the proximal line of excision without evidence of lymph-node metastasis. The interval of time following resection until symptoms reassert themselves is thus designated as the silent interval, the recurring symptoms due essentially to actual recurrence or continued growth of the malignant cells within the gastric pouch or the anastomotic line and not due to recurrent lymph-node involvement. Six such patients have been studied, and the silent interval in each has been determined. Those observations constitute the basis of this presentation. The survival time as well as the manner in which recognition of the recurrence was noted is presented. The case records are taken largely from the material studied by State, et al. in their 10-year survey (1936 to 1945, inclusive) of the results of surgical treatment of carcinoma of the stomach at the University of Minnesota Hospitals. One additional case has been added.

The data concerning these patients is presented in table 1. It will be noted that the silent interval in six cases ranges from 10 to 30 months, the average interval being 20 months. No symptoms referable to the stomach were present in these patients during that interval. The symptoms which reasserted themselves following the silent interval consisted essentially of the same symptoms as were elicited before the time of the gastric resection. Prior to resection, the complaints were almost uniformly that of epigastric distress and weakness; occasional nausea and vomiting occurred in two of the patients. Following the silent interval, the former symptoms recurred with an added element of fullness after meals and a decreasing ability to take food, elements suggestive of partial stomal obstruction in a gastric pouch of limited capacity.

The actual survival time in five patients succumbing from recurrent carcinoma at the anastomosis in the gastric pouch ranged from 15 to 28 months with an average of 21.8 months. The proximal line of resection was involved microscopically in all cases, and no lymph-node metastases were noted, save in 1 instance, where 1 node contained some metastatic carcinoma. This case was included in the study because it was felt that the recurrence in this patient represented the continued growth of the malignant cells in the line of excision. All cases were classified into Borrmann Types III and IV pathologically. It is interesting to note that the shorter silent intervals occur in the two patients in which a 98-percent resection was carried out. It is possible, in these circumstances, that the recurrent carcinoma in the smaller gastric pouch produced symptoms at an earlier date because of the reduced capacity of the pouch, a smaller space-occupying tumor producing relatively greater or earlier disturbance in the continuity of the gastrointestinal tract. It is notable, conversely,

TABLE 1.—*Study of silent interval following subtotal gastric resection in patients with gastric cancer in which residual tumor was left in the proximal line of resection; no (or minimal) lymph-node metastases at time of resection*

Patient	Age	Hospital No.	Percent resection	Weight of removed stomach	Involvement		Silent interval	Interval until death	Recurrence noted
					Line of resection	Nodes involved			
							Months	*Months*	
A. W.	57	642360	80	----	+	0	23	24	By X-ray.
F. R.	55	715046	85	----	+	1	19	20	Palpable mass.
O. A.	60	721380	98	390	+	0	17	22	Rectal stricture.
C. B.	68	732161	98	315	+	0	10	15	Epigastric mass.
C. D.	49	721354	90	450	+	0	21	28	By gastroscopy.
E. W.	66	795848	70	----	+	0	30	(1)	By X-ray.
Average	----	----	----	----	----	----	20	21. 8	

[1] This patient was reoperated upon, and a recurrent tumor of the gastric pouch at the anastomotic line with loca involvement of surrounding structures was excised *en bloc*, and gastrointestinal continuity was reestablished.

that one patient (E. W., Univ. Hosp. No. 795848) in whom a 70-percent resection was performed for a large ulcerating malignancy of the stomach enjoyed a period of 30 months freedom from symptoms. Moreover, symptoms were minimal for an additional 15 months at which time X-ray examination revealed a recurrence at the site of anastomosis. This patient was reoperated upon 45 months after the original resection and was found to have an adenocarcinoma involving the distal gastric pouch at the anastomotic line, the previous gastrojejunostomy site, invading the mesocolon, transverse colon, and a small portion of the superior margin of the pancreas. The mass was excised *en bloc* to the superior mesenteric vessels, and gastrointestinal continuity was reestablished after excision of segments of stomach, jejunem, and colon by gastrojejunostomy, jejuno-jejunostomy, and colocolostomy. The anastomoses were performed employing the aseptic technique. All lines of excision of the re-resection were free of tumor cells as were lymph nodes removed. The patient recovered without event and was discharged from the hospital on the seventh postoperative day. The latter resection is too recent to permit follow-up observations other than that the patient is symptom-free at the present time, 6 weeks from the time of surgery.

No other patients in this group, other than the one just discussed, were subjected to re-operation when recurrence was noted. One patient (C. D., Univ. Hosp. No. 721354) was readmitted to the hospital expressly for the purpose of re-operation; several bouts of pneumonia, however, ultimately prevented adequate preparation for operation, no re-exploration being done. Two patients (F. R., Univ. Hosp. No. 715046 and C. B., Univ. Hosp. No. 721380) were thought to have recurrent masses too large for re-excision. One patient (O. A., Univ. Hosp. No. 721380) had evidence of distant metastases, a nonulcerating rectal stricture, as well as recurrent

tumor in the residual gastric pouch. And, finally, one patient (A. W., Univ. Hosp. No. 642360) presented evidence of recurrence at a time (1938, 2 years after subtotal resection) when re-excision and total gastrectomy carried a higher mortality rate than is experienced at the present time. With the decreasing mortality rate associated with the improvement in surgical technique in the performance of total gastrectomy, it is justifiable to suggest that patients in whom malignant cells remain in the proximal line of excision during and after subtotal resection be subjected to more extensive resection. Moreover, it may be proper to perform total gastrectomy in all patients with gastric carcinoma of Borrmann Types III and IV. Re-resection should be carried out early while the patient is in good health, preferably well within the minimum silent interval, and, ideally, at the time of the initial operation if it is noted at the time that tumor cells remain in the line of subtotal excision.

From the data presented, it will be noted that the average silent interval of 20 months in 6 cases and the average survival time of 21.8 months in 5 cases roughly corresponds to the average survival time of 22.4 months in all patients subjected to gastric resection and who died of gastric cancer as reported by State, Moore, and Wangensteen. It is probable that in their survey, cases with lymph-node involvement at the time of resection may present a shorter interval of survival than when grouped together with cases showing no lymph-node metastasis.

In conclusion, a study of the case records of six patients in whom a subtotal gastric resection was performed for gastric carcinoma and in whom cancerous cells remained in the proximal line of excision without evidence of lymph-node metastases revealed that these patients were free of symptoms for an average period of 20 months following resection; this period is designated as the silent interval.

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Chairman Palmer: Thank you, Dr. Friesen. I think I will ask Dr. George E. Moore if he wouldn't like to open the discussion of these two papers.

Dr. Moore: In the first place, I would like to say that I think Dr. Meissner's paper is very apt. We agree wholeheartedly with his conclusions. The results of a similar study by the Minnesota group appeared in November issue of Surgery, Gynecology, and Obstetrics, Vol. 87: 513-518 (1948).

As a result of this study I feel that I must differ with the people who have attempted to prognosticate the survival of patients with gastric carcinoma either by Borrmann typing or microscopic gradation of the lesion. The former, of course, proposed by Dr. Schindler, and the second, by Broders. As far as the Borrmann classification is concerned, we found that there is no strict statistical correlation between Borrmann type and survival. The Borrmann type does affect the prognosis of groups of cases to the extent that lesions of types I and II provide a proportionately larger number of cases free of metastasis, the real determinant of survival being the presence or absence of involved nodes.

We have, at the present time, three patients with Borrmann type IV lesions, who have survived 5 years following gastrectomy. We have two others who have survived 3 years and have not yet been exposed to a 5-year period. We have one other patient, a doctor, an M. D. from Minnesota, who had a typical infiltration type IV carcinoma, but with no positive nodes, which was resected. He died this June, 13 days before he reached his fifth year. He died of a cerebral vascular accident.

We did find a very good statistical correlation between the metastasis and survival. That is to say, that of our small group of patients (100), 71.4 percent of the patients with negative nodes lived 3 years, while only about 17.7 percent of those with positive nodes lived 3 years.

As the time interval increases, the difference is even more significant. At the present time, of those patients with positive nodes only about 9 percent are alive, while of those without nodes, the percentage alive is approximately 47. It cannot be said that the difference is due to selection, since the distribution of Borrmann types in our series of cases is very close to that of Dr. Schindler's series.

I might add that I do not agree with Dr. Meissner, that he was justified in using kodachrome slides as an aid in classifying the Borrmann type for those lesions which weren't available for study. As he probably knows, Dr. Borrmann originally not only examined the lesions but he also cut across the diameter of the lesion and examined the cut surface to see whether they infiltrated or not, grossly, of course.

As far as Dr. Friesen's paper is concerned, I have very little to add. I think it is important, in view of some of the work going on, to realize that there is a long silent interval before a gastric cancer grows to such size as to provoke symptoms.

Chairman Palmer: Thank you, Dr. Moore. In defense of that large clinic in a small town in Minnesota, I might point out that if one reads figures, there is a pretty definite trend, but as Dr. Moore has pointed out so very well, there are many exceptions to be noted, even in those figures. Dr. Shields Warren, won't you continue the discussion?

Dr. Warren: I would like to develop further two points touched on by Dr. Meissner and Dr. Friesen. First, is that the results of therapy of gastric carcinoma are admittedly poorer than of any other lesion. It is perhaps significant that in gastric carcinoma the principles of surgical therapy of carcinoma are more frequently violated than in any other.

No one would dream of taking off two-thirds of a breast for a carcinoma of the breast. I think that perhaps the remarkable fact is that we get as many cures as we do, and I am quite sure, in view of the studies that have been made of the types of recurrence that Dr. Friesen has presented, that the best and simplest way to get better results in the treatment of cancer of the stomach is to do total gastrectomies, to treat a cancer of the stomach as we would treat cancer in any other locality.

With regard to the matter of blood vessel invasion that Dr. Meissner brought out, it is important to remember that contrary to what we were taught in second year

pathology, a good many epithelial tumors have the habit of invading veins and can spread by the venous drainage without involving intervening lymph nodes, and I think some of the failures where there is neither lymph-node involvement at the time of removal of the lesion nor evidence of extension to the line of resection probably is explained by these cases of blood-vessel invasion.

I think it would be well if pathologists looked more frequently for this evidence of spread. I think we can not reiterate too often the fact that cancer of the stomach resembles cancer of other organs in that it is very rarely originating in normal tissue; one of the points that gives us diagnostic hope is that the weight of evidence indicates that there has to be abnormality preceding many frank gastric lesions, and for a considerable time prior to the development of the full-blown carcinoma.

The studies that have been made thus far have not led, it is true, to an infallible diagnostic test, but the fact that these differences exist should encourage us to keep on trying and to hope that we may get some way, concentrating on cases that are still early and capable of therapy. But even in an early case, as soon as one attempts to compromise what is convenient to do with the habits of carcinoma, one gets into grief. The only type of safe procedure, I am inclined to believe, is to do all one can whenever one can.

Chairman Palmer: Thank you, Dr. Warren. I think these two papers should now be open for general discussion. Dr. Schindler.

Dr. Schindler: I have a question to ask Dr. Moore. I was naturally very much gratified to hear Dr. Friesen say that type III and type IV carcinomas should have a total resection, not a partial one, and Dr. Warren now has pointed out that total gastrectomies are the only solution to the problem of gastric carcinoma. I would even go so far as to include type II carcinoma in those in which a total gastrectomy should be done. In our experience it is only type I in which frequent cures are obtained by partial resection. Dr. Moore's results do not agree with my own statistics, and I wonder now many of his cured type IV carcinomas had a very large if not a total gastrectomy.

Chairman Palmer: Dr. Moore.

Dr. Moore: Usually about 80 to 95 percent resections are done. I believe that less than 10 of the cases in this series were total gastrectomies. If, by gastroscopy or other methods, you say a certain group is not worthwhile operating on, a certain number of people will lose their lives who otherwise would not if they were resected.

Chairman Palmer: If I understood your original remarks correctly, Dr. Moore, it was not that you were disagreeing with the premise that Borrmann type I is, shall I say, a much less malignant one than Borrmann type IV, but you were, rather, emphasizing the fact that there are Borrmann types IV which can be saved?

Dr. Moore: That is correct. In other words, however small the numbers involved of these patients, they are worthwhile saving. When we complete our large screening tests such as routine X-rays of the stomach, we may pick up only 1, 2, or 3 patients per thousand, and even some of these cases are inoperable. In contrast, a series such as ours involving 100 patients with 67 patients having Borrmann types III and IV; 7 patients survived 5 years, and 6 others were alive and well but had not yet been exposed to a 5-year period. Thus, the number of 5-year cures effected by resecting lesions of an unfavorable type is much larger than can be salvaged by extensive X-ray surveys.

Chairman Palmer: Dr. Shimkin.

Dr. Shimkin: I would like to make a comment and ask some questions. I was impressed by Dr. Friesen's figures on the survival rate of patients who had resections of the local disease in face of liver metastases. When resection was done, the survival period was 15 months, whereas a similar group in whom no resection was done, the survival period was 5 months. The apparent conclusion is that if the local lesion is resected, despite distant metastases, the survival of patients is longer, 3 times as long, in fact, from Dr. Friesen's data. Of course, an inevitable element of selection must

be involved; patients who had resections were those with less advanced local lesions that made the procedure feasible.

The first question is: Is it the concensus of the surgeons here that, when feasible, local gastric or rectal carcinoma should be resected despite metastatic spread found at operation? This question constantly arises before consultative tumor boards.

The second question is: Are there any factual data to support the following two clinical impressions? The first is that metastases in the liver or other distant sites seem to progress slower when primary gastric or rectal carcinomas are removed. Second, that when such primary growths are removed in the face of metastatic spread, the patient often survives for some period in relative comfort and well-being, and then expires rather suddenly, as if from some decompensation.

Chairman Palmer: Anything further on the points raised by Dr. Shimkin? Dr. Pack, of New York.

Dr. Pack: Apropos of the remarks made by Dr. Shimkin, I wish to emphasize that the true measure of palliation for gastric cancer is not merely the duration of postoperative life. There is nothing to be gained in enabling the patient to live longer and suffer more, as Dr. Will Mayo said many years ago. The important evaluation of palliative relief is in the degree of comfort and relief of pain afforded the patient during the remaining months of life. I believe that gastrectomy even in the presence of small hepatic metastases is a worthwhile palliative procedure. Our time as surgeon is not so important as to be reserved only for the patient with a likely prospect of cure; the hour or two required for palliative resection of an incurable cancer is richly rewarded by the gift of months of comfort and good digestion for the patient.

I dislike to disagree with my friend, Dr. Shields Warren, but pathologists are notorious iconoclasts and pessimists; they do too much surgery in the postmortem laboratory. His statement that progress in the surgical treatment of gastric cancer is the gloomiest when compared with the outlook for other cancers, should be challenged. The prognosis for cancer of the stomach is infinitely better than for cancer of the esophagus, bone, brain and pancreas. As a clinician whose major effort during the past 20 years has been the study of gastric cancer, I am certain about the great improvement in early diagnosis, a higher percentage of cancers in the favorable group, an increased rate of resectability, a lowered operative mortality, a tremendous betterment in postoperative management and a higher rate of definitive cure. Our 5 year care rate for resectable gastric cancer is now 34.5 percent. A decade ago all patients with cancer extending to and involving the gastric cardia were considered hopeless, but now 38 percent of all gastric resections are of the radical type, namely, total gastrectomy or transthoracic esophagogastrectomy. A physician who devotes his entire time to cancer therapy, of course, must be an optimist by nature, else he should abandon this work and become an obstetrician, who divorces mother and babe with joyful end results and disproportionate credit.

I question the conclusion as to the time necessary for the evolution of gastric cancer based on information about the silent interval of time elapsing between incomplete resection for gastric cancer and the onset of symptoms and signs indicating that the residual cancer had reached a clinical stage in its growth. In my opinion, this is not of fundamental importance. It certainly cannot be assumed to represent the length of time necessary for a latent primary cancer to develop. The stated average of 22 months elapsing between gastrectomy and transsection through actively growing cancer and the subsequent appearance of symptoms does furnish three things: (1) an estimate of the expected rate of growth of active gastric cancer, (2) the opportunity for the surgeon to make up his mind when he will do the necessary second operation, and (3) sufficient time to pluck up courage to approach the patient with the statement that he must undergo a second operation because of incomplete excision. We have made it a rule at the Memorial Hospital to request frozen section analyses of the lines of transsection for cancers extending into the esophagus, so that we can decide if the removal is adequate before we start the anastomosis. We are of the opinion that

if the pathologist does give us a report of residual gastric cancer at the line of incision when the fixed tissue studies are completed several days later, the time to operate is as soon as the patient can again be prepared, rather than to wait expectantly for the inevitable recurrence.

We would like to know, however, the approximate time required for a gastric carcinoma in situ to evolute to the stage where it provokes symptoms, is visible roentgenographically or is clinically identifiable as such. We found 1 key case last year that may give us this opportunity to study such a latent period. In a group of 1,700 supposedly normal individuals subjected to gastrointestinal X-ray studies at the Memorial Hospital by Dr. Robert Sherman, 1 esophageal leiomyoma and 2 gastric cancers were detected and successfully resected. One aged patient, well beyond the Biblically allotted threescore years and 10 and a poor operative risk, had 75 percent of her stomach removed for a small sessile cancer barely visible on fluoroscopy. Close inspection of the gastric mucosa revealed 15 or more tiny white flecks scattered diffusely in all parts of the resected specimen. Microscopical study of these macular spots showed multicentric tiny carcinomas in situ. There were many other small foci not grossly seen but discovered on microscopical study of apparently normal mucosa. Under ordinary circumstances, we would have reoperated on this patient and removed the residue of stomach; i. e., completed a total gastrectomy. Undoubtedly there are some cancers in situ in the remaining portion of her stomach. On her promise to cooperate and because of her poor operative risk, we elected to maintain her under close surveillance. The operation was done in June 1947, and there has been no suspicious evidence to date (October 20, 1949) after a lapse of 2½ years. If a clinical cancer ultimately develops in the proximal segment of stomach, we will have some inkling about the evolutionary period of gastric carcinoma in situ.

Chairman Palmer: Thank you, Dr. Pack. In order that we may have a little time left this morning for general discussion of the whole program, I think I will conclude this portion of the discussion, first asking Dr. Meissner if he has any additional remarks to make.

Dr. Meissner: I believe all the questions have been answered already.

Chairman Palmer: Dr. Meissner thinks the questions have been answered already. Dr. Friesen?

Dr. Friesen: I want to thank those who discussed the program.

Chairman Palmer: Thank you very much. We will have a 5-minute recess, after which we will have general discussion of the entire symposium which will be conducted by Dr. Wangensteen and Dr. Ivy.

(A short recess was taken. The conference continued with Dr. Wangensteen presiding.)

Dr. Wangensteen: Dr. Stone made a remark last night asking to whom we were accountable. This is the fourth gastric cancer conference that has been held. We understand life in looking backward. Unfortunately, we have to live it looking forward.

I assume Dr. Stone might have said that in the ultimate analysis we are accountable to ourselves. I think he was a prejudiced witness in his own behalf. I suppose it would be a little unfair to ask ourselves whether or not we have been good and faithful servants in this public accounting of accomplishments in the field of gastric cancer. I assume the main thing we have accomplished is to stir up interest in the general problem. You can see from the discussions that there is real interest.

Some of you have been attending these gastric cancer conferences for 10 years. I have not. During those 10 years some experimentalists have learned how to create gastric cancer. We are all hopeful that presently there will be much information available relating to both extrinsic and intrinsic factors which modify the incidence and occurrence of such experimentally induced cancers. Certainly, the creation of the cancer in the stomach is by no means our objective, satisfying at the moment as that attainment may be.

The complexion of this group is a very complex one. Though I live in Minnesota, I refer to a very distinguished New England poet, the lines of whose poetry are music;

there is music in every line. He wrote a very fine poem we all love called "Evangeline." When I think of Longfellow writing "Evangeline," describing the pursuit of Gabriel, one for the other, all around, up and down the bayous of the Mississippi River, I don't think he was thinking particularly of a physical search one for the other, but what Longfellow was telling us was to try to match moods while there is still time.

I don't know in this complex structure where we are to match moods in the problem of gastric cancer. Possibly the question of intrinsic or extrinsic factors would command the interest of most experimentalists and clinicians for purposes of this discussion.

From the present status of our knowledge, any inferences which we might make with reference to the importance of either of these factors would be largely speculative. Ultimately a good treatment comes from speculation. Once a crucial experiment is constructed, we are more likely to secure a satisfactory answer, and I can understand why John Hunter said to Edward Jenner, "Don't think, try the experiment." Well, we are all grappling and wrestling with this question and trying to grasp the problem with a handle which will help us to shake it down so something will come out of it.

We have had these four gastric cancer conferences and a lot of interest has come out of them, and interest is what makes burdens light. Those of you who have been consumed by interest know that it makes the burden perhaps just one-thousandth as heavy as it was when there was no real interest in the character of that burden. We have plenty of interest, but we need handles to help shake this problem down. We would like to know just what cancer is. It is easy to ask rhetorical questions. A child can ask questions that we can't answer. Cancer—what is it? Some day maybe we hope to know. What are the progenitors of cancer?

We have heard a lot of discussion on some phases of that today. No one has been so bold as to say, Can we prevent the development of these precursors? Can we stop them? We are, in a sense, like a drowning man. We don't know very much about those things though we say to ourselves, "Well, can we recognize cancer when we have it?" In other words, while we are pondering what cancer is, and what its precursors may be, we are forced often by the urgency of circumstance to ask, "May we, or members of our family, or our associates have cancer?" And so, too, with a drowning man. Thoughts of saving his life preoccupy his mind to the exclusion of considerations concerning techniques of swimming. There are even those prophets among us who say it isn't even worthwhile trying to diagnose cancer before the onset of symptoms. Then, finally, we say, "Well, can you cure it when it is obvious that I have it?" I think it would be fair to say that we are confronted by a real problem accountable for the enthusiasm of the two surgeons who have just spoken. I suppose if all the surgeons had spoken, there wouldn't be any time for the rest of you to say anything.

Yes, the surgeons have some right to their enthusiasm. If we had made equal progress in every chapter of gastric cancer to that made by surgeons, we would have some reason for feeling reasonably satisfied with our accomplishments. My colleagues and I have been discussing for a few months now the practicality of intervening again surgically 4 to 6 months after the initial operation in group C cases, in whom the lymph nodes were found to be involved af the time of the first operation. We are proposing to begin with colic cancers extending such reentries of the abdomen to gastric and colic cancer as well. I believe we may cure some of the patients presenting lymph-node-involvement by this means. In any case, we are planning to embark on the scheme presently. As the presentation of Dr. Friesen suggested the usual length of the silent interval is in the area of 20 months. If reexploration is undertaken then, the cancer will be late if deposits remained in some lymph nodes.

As Dr. Warren said earlier, there is no real accomplishment yet in the field of gastric cancer. As he intimated, hope is the last refuge of the discouraged. Nevertheless we must not lose hope. If those who take it upon themselves to speak for us say, "Well, results are bad; the patients who have symptoms do not get anywhere; we will not get anywhere examining the general population; it is a fruitless, useless task." How can we face ourselves let alone the public who expects from us that we do our best?

That is why the National Advisory Cancer Council has given us this privilege and this opportunity to see what we can do with this problem. Moreover, hope is a parent to faith and it is only as we can keep both faith and hope alive that we can take courage to continue to go forward. It is necessary that we turn the cancer problem around in our minds and look at its many facets, that we try to match moods in this problem with other cancer workers and eventually something may come from our labors.

Therefore, with the thought of trying to focus discussion on a limited area, I would suggest that in the concluding moments which are available to us (and none of us can speak for long) we try to address ourselves to this problem: What are the things that speak for intrinsic or extrinsic relationships or factors in the gastric cancer problem?

I don't want to paraphrase Shakespeare who had Cassius say,

> The fault, dear Brutus, is not in our stars
> But in ourselves, that we are underlings.

If our problem is that simple, this is an intrinsic problem. We should address ourselves to it. Yet, there are so many things, certainly, in the clinical picture of cancer which suggest to most of us that environmental factors are important, too.

I shall ask Dr. Lorenz if he wishes to amplify that statement which he made yesterday.

Dr. Lorenz: I want to emphasize again that with the different carcinogenic preparations we administered in place of drinking water we have not been able to produce any change whatsoever in the glandular stomach. In addition, in those mice that spontaneously show a hyperplastic lesion of the glandular stomach after the age of 8 months, strain I and strain dba, these lesions remained unchanged by the carcinogenic agents.

In other experiments we produced ulcers of the glandular stomach in the hope that the orally administered carcinogens might adhere at the site of the lesion. The ulcers were induced either by giving acid pepsin solution or by interrupted exposure to low pressure (equivalent to about 25,000 feet altitude). No lesions beside the ulcers were observed in the glandular stomach. Lesions of the forestomach were found. Since carcinoma in the forestomach develops rapidly and kills the animal eventually, the question arises as to whether or not the experiments could be carried on long enough to induce any changes in the glandular stomach.

Guinea pigs were given suspension of methylcholanthrene in place of drinking water for over 2 years. During that time over 1 gram of the hydrocarbon was ingested. No changes were induced in the glandular stomach. The majority of these guinea pigs eventually died of the cirrhosis of the liver, which was probably induced by the injection of the carcinogen.

From all these experiments, the conclusion was reached that perhaps we should not look for an extrinsic factor in the food in the induction of carcinoma of the glandular stomach but perhaps include an intrinsic factor which may be present in the blood stream.

Chairman Wangensteen: Does anyone want to address himself to the fact that extrinsic factors are important? (No response) Dr. Hitchcock, would you like to show your slides?

Dr. Hitchcock: Lately we have been interested in reviving a classical experiment at the University of Minnesota that was performed at the turn of the century and thereafter for about 14 years in Copenhagen by Professor Fibiger at the University there. At that time the interest in experimental carcinogenesis was placed on parasites that infested various parts of the body.

In pursuing his work for many years, Professor Fibiger was able to produce carcinoma, squamous carcinoma, that is, in the forestomach of all types of rats, including the then available tame laboratory rats and wild rats of various types. The incidence of his cancer was 54 percent; 35 percent of his total lesions had metastases primarily in the retroperitoneal lymph nodes and also in the lung. Subsequently, when the study of the vitamins had its advent, it was shown that a good deal of the cause in this work was probably due to a vitamin A deficiency. Subsequent workers, including Bullock and

Rohdenburg in the East, Papenheimer and Larrimore, Cramer in England, and Passey, Leese, and Knox in England, at various times worked on this problem, and none of them was able, apparently, to produce the type of lesions that Dr. Fibiger got.

Now, fortuitously, we discovered the existence of the parasitic nematode of Gongylonema neoplasticum in wild rats in our community outside the city of Minneapolis, and at the present time we have set up experiments and are using Sprague Dawley strain of rats as well as wild Norwegian brown rats that we are catching in the community, and if you would permit me I should like to show you three slides that I brought which will demonstrate the type of lesions that we are getting with both the spiroptera infestation and vitamin-A deficient diets. (Slide.) This first slide is the stomach of a wild rat, the Norwegian brown rat, caught in the community, and in which the parasitic nematode or Gongylonema neoplasticum is present. The animal is on free forage diet, that is, the diet obtained out in the regions of the rendering plant where he was trapped. I would determine from the basis of the type of material that is available for him to eat that his diet is essentially normal in all respects and, yet, you can see that there is a marked thickening, a hyperplasia, and hyperkeratosis with some papillomatosis in the squamous portion of the stomach.

(Second slide.) This is one of my laboratory rats that went for 66 days on a vitamin-A deficient diet only. There were no parasites present here. The squamous stomach is on the right hand of the specimen, and you will note that it is thicker than a normal stomach. It is hyperplastic, with some hyperkeratosis.

(Third slide.) This stomach is one of the Sprague Dawley strain of laboratory rats that was infested with the parasite by feeding it two periplanata americana cockroaches that were heavily infected with parasites. Thirty-five days after the parasites were induced into the animal, the animal died. He had been on a vitamin-A deficient diet for 66 days, and this is the result that we see in the squamous forestomach. I should like you to note the tremendous hyperkeratosis that is present, the massive thickening of the squamous epithelium, and blockage of the lumen. In microscopic sections we see the thickening of the cornified squame cells and epithelial downgrowths to the muscularis.

(Fourth slide.) I should like you to notice the esophagus in the picture. It is obstructed completely with the excessive squamous elements. The parasite is present here also. The problem, we think, is very interesting, and we are attacking it from the standpoint mainly of nutrition, utilizing the parasite as perhaps a good medium for getting malignancies on which we can evaluate the various nutritional deficiencies. At least we are very hopeful that on the basis of these few specimens our strains of rats and our method will prove adequate.

Chairman Wangensteen: Many of you, of course, have had experience with Fibiger's experiments, and it is difficult for me, as a clinician who has been interested primarily in some of these so-called polyp precursors of gastric cancer, to deny that a lesion such as Dr. Hitchcock produced within 50 short days may not have some relation to the cancer, once we get the animals to live longer. When Dr. Peacock was here 2 years ago, he talked at great length on this subject. There is no proof yet, but it is ground I think which both clinicians and experimentalists could till with some profit,

There was a paper on this the other day by Dr. Copeland. Does anyone want to speak further on any dietary relation to gastric cancer?

Dr. Nelson: I would like to call attention to the work of Robert McCarrison in India between 1910 and 1920. He fed monkeys deficient diets and was able to produce marked atrophic, and in some even necrotic, changes in the adrenal glands. These same animals on prolonged deficiency diets developed atrophy of the gastric mucosa.

I wonder if these atrophic changes in the adrenals and other endocrine glands may not be related to some of the intrinsic factors in the production of cancer.

Chairman Wangensteen: Dr. Fern Smith yesterday spoke of the intrinsic factor, the hormonal factor. She found an increase in incidence with methylcholanthrene type of cancer following removal of the sex glands. I suppose she is contemplating an additional influence of what she took away to see if more than is ordinarily present would be helpful in inhibiting tumor.

Does anyone want to discuss the matter of the relationships of the hormonal influence to gastric cancer?

Dr. Stewart: I might amplify some of the remarks that Dr. Lorenz made. In our study of the oral administration of carcinogenic hydrocarbons some 500 mice have been treated in this way. No adenocarcinoma of the stomach has occurred in any case. It may be that the carcinogen is not absorbed by the glandular stomach or does not come in contact with the glandular mucosa, owing to the mucus barrier. In the small intestines, when the carcinogen is given mixed with the food or in the olive oil which is digested and the carcinogen liberated so that it may be absorbed, then we find carcinoma of the small intestine, Moreover, along the route of absorption we find mesenteric retroperitoneal and pulmonary tumors. In patients with these tumors, the possibility that they may be due to ingested carcinogen is rarely considered. I think our work might suggest exploration along this line,

Chairman Wangensteen: With reference to what Dr. Stewart has just said, we might be mindful of what Dr. Peacock said 2 years ago when the gastric conference met. I recall he addressed a question to one of the chemists in the room, inquiring how many molecules there were in that one-hundredth milligram of methylcholanthrene they had used, the smallest amount they had used to produce a cancer. He was told one-thousand-million-million. In a dose you might have a powerful carcinogen. Dr. Lorenz.

Dr. Lorenz: I might add here that the amounts of ingested carcinogen are quite high. In mice they reach several hundred milligrams per animal, and in guinea pigs over 1 gm. As to the forestomach lesion in control mice such as hyperkeratosis or papillomatosis, I want to point out that they can easily be produced by the administration of Aerosol solution or mineral-oil emulsion. Often the whole forestomach mucosa may be covered with papillomas, yet never a change to malignancy was observed. These lesions may be due to a deficiency in vitamin A produced by the Aerosol solution or the mineral-oil emulsion.

Chairman Wagensteen: One thing I think should be said at a conference like this is that we are all perfectionists in a sense; we like to come out with a nice answer. Often we have to be satisfied with something far less. I do not believe that clinicians would find much fault with your ability to produce a cancer any place that is a cancer. In other words, I think this whole argument of whether it is in the forestomach or in the true glandular stomach should cease because we do not yet know the threshold to the production of injury, ulcer on one hand or cancer on the other hand. We are just beginning to learn how very sensitive the esophageal mucosa is to ulcer production. If the pylorus of the dog is obstructed and histamine-in-beeswax is given, esophagitis is observed in 100 percent and evidences of gastritis in none. In fact, within a period of 4 days, perforation of the esophagus will be observed in 50 percent of the dogs, while the stomach remains normal. The breaking strength of the dog's normal stomach is in the order of magnitude of 80 mm. of mercury, while that of the esophagus is 900 mm. of mercury.

In the light of all this, it is surprising that a person develops an acid-peptic gastric ulcer at all. Certainly, there are other antecedents of the ulcer than gastric peptic digestion.

In the situation described by Dr. Hollander, such studies will help us understand how the acid-peptic juice effects the first beginnings of erosions, but the whole question of vulnerability, I think, will inject itself very palpably into the whole question.

I think we should stop making sharp distinctions concerning whether the cancer is in this or that portion of the stomach until we have more factual information about the relative susceptibility of various areas in the stomach to experimental carcinogens. There are undoubtedly differences in threshold to cancer susceptibility, We need more factual information on that tissue susceptibility.

I would like to call on Dr. Gomori. I know he has some techniques which might be helpful to experimentalists in this field.

Dr. Gomori: I have to apologize to this group for bringing up a subject that does not

properly belong under the heading of general discussion, but I think it is sufficiently interesting to be shown.

About 2 months ago, in the course of histochemical experiments on the substrate specificity of phosphatases, it was found that 1 out of the 19 substrates used produced a pattern of enzyme distribution in tissues entirely different from the other 18 substrates. This substrate, the chloroanilide of phosphoric acid, is not a phosphoric ester; it is substituted phosphamide, and it was included among the substrates because of previous reports on the existence of an enzyme which hydrolyzes this type of linkage between nitrogen and phosphorus.

The important feature in the distribution of enzyme, when this substrate was used, is this: It is present in minimal or moderate amounts in almost all tissues, but it is always present in large amounts at 2 sites, first in the nervous system, and second in malignant epithelial tumors. So far I have examined about 70 malignant tumors, and in 100 percent of the carcinomas the specific staining of the malignant element was obtained, whereas in sarcomas the percentage was much lower. I would like to show you a few of my slides (first slide).

The enzymatic reaction shows up in a brown shade. This is a section of a cancer of the uterine cervix. As you see, there is a very sharp line of division here (indicating), and only the malignant portion is stained and not the benign portion. This is just one type of distribution. By the way, the high grade of malignancy of this tumor was shown by widespread metastases.

(Second slide.) This is a squamous cancer of the skin in which the invading epithelium is distinctly brown, showing the reaction in the tumor and away from it in the epithelial pegs up to a certain distance; this area, often called the area of unrest by dermatologists, is also stained. From here on (indicating), however, there is no staining whatsoever. This type of distribution is often observed around carcinomas, i.e., the reaction extends 1 or 2 mm. beyond the actual confines of the lesion.

(Third slide.) This is a carcinoma of the breast of the scirrhous type. Here (indicating) is a duct which is absolutely unstained. Around there the nest of carcinoma cells shows an intense reaction.

(Fourth slide.) The next slide is a carcinoma of the colon; normal mucosa here (indicating), practically unstained. All of the invading neoplastic glands are intensely stained.

(Fifth slide.) The next slide is a carcinoma of the stomach. Up here (indicating) is the more or less normal mucosa. Here is the border (indicating), already showing staining, and here the invading malignant glands are almost black.

(Sixth slide.) This is a very peculiar carcinoma of the stomach, of which I have seen only one example. It was composed of two entirely different elements. The first part was a more or less benign looking papilloma and, interspersed in the connective tissue stroma, there were nests of rather large cells, arranged in a haphazard way somewhat reminiscent of a hypernephroma.

(Seventh slide.) This is a carcinoma of the stomach of the scirrhous type. There was only a very small ulcer present, and this section was taken over 5 cm. from the edge of the ulcer. You see, the normal mucosa is unstained, but in the submucosa groups of invading malignant cells are intensely stained. This is very far away from the primary lesion, about 10 cm., close to the line of resection. Here (indicating) the mucosa and the submucosa are infiltrated by groups of cells and even single cells, of which every single one is outlined by the reaction.

(Eighth slide.) This is the floor of a rather large ulcer in a case of scirrhous carcinoma of the stomach. Again you find that connective tissue framework is unstained, but every single malignant cell stands out very clearly.

Unfortunately, I do not expect that this new reaction will contribute much to the diagnosis of malignancy, because if you happen to have a tumor that shows various degrees of malignancy in the same slide, the intensity of staining will parallel the morphological appearance. Semimalignant parts will stain slightly; moderately malignant ones medium; and the very malignant ones dark. The stain cannot be used

on smears. The enzyme seems to be highly soluble, and as soon as the cells are suspended in any medium, the enzyme will diffuse out. However, we are working now on a quantitative chemical demonstration of this enzyme both in tissue extracts and in natural secretions and in serum, but I don't have any data to report yet.

Chairman Wangensteen: Dr. Brunschwig has a question to ask.

Dr. Brunschwig: Have you this on embryonic tissue?

Dr. Gomori: Yes, findings are variable.

Dr. Brunschwig: What about the whole fetus of a rat at about 10 days?

Dr. Gomori: Whatever happens to be growing fast at that embryonic stage will stain intensely. Epithelial organs, in general, stain very intensely.

Chairman Wangensteen: Dr. Morgan made a novel suggestion to me yesterday evening about combining knowledge of chemistry with roentgenology to simplify, although it might intensify, the labor of the roentgenologist with reference to the achlorhydric patients who may harbor gastric cancer. Is Dr. Morgan still here?

Dr. Morgan: The material which was presented by Dr. Roach yesterday and again referred to by Dr. Rigler last night may not be the final answer to the question of the early diagnosis of gastric cancer. However, it does seem at least to be a step in the right direction.

Dr. State of Dr. Wangensteen's group in Minnesota, in a discussion before this meeting, raised the question as to the feasibility of combining the chemical studies that have been performed by workers in his group and the roentgen work, which many people are working on now, into a test which might be considerably better as a screening procedure than the procedures used up to the present time. Dr. State suggested the possibility of developing a capsule, including barium, which might be swallowed by the subjects under test and arranged so that the capsule material dissolves at various pH values. It is entirely possible that this test might prove beneficial as a screening procedure inasmuch as Dr. Rigler has shown that there seems to be a rather distinctive correlation between gastric carcinoma and achlorhydria. Whether or not such a test might be feasible I don't know since there is a good deal of question as to whether or not the capsule which would dissolve at various pH's would be as good as one which might dissolve at various degrees of acidity. However, a simple procedure that would combine the testing of gastric acidity and a roentgen examination of the stomach has intriguing possibilities.

There is one other question, while I am on my feet, that I would like to discuss. This concerns something that Dr. Newell said in response to Dr. Rigler's paper last night. He was quite pessimistic about this whole problem of survey work in gastric carcinoma. We at Hopkins, and fortunately workers in many other places, don't have so pessimistic an outlook. Dr. Newell was particularly impressed with the economic difficulties of gastric surveys. However, if you look at the facts of the matter, the situation, I don't think, is quite as pessimistic as was indicated last night.

To give a few statistics, the incidence of carcinoma at the age above 40 is about 1 in 800. Although our surveys are not altogether a good index, it seems that the cost is somewhere between one and two dollars a patient. That means that the average carcinoma, if found, would cost around $1,000 or more. I think everybody must agree that $1,000 isn't too much to pay for adding 20 years of life to a patient, or to anybody, especially when that individual is close to home. Certainly, in these days, when the average individual on the street thinks nothing of going out and paying $2,000 for an automobile that lasts 3 or 4 years, it doesn't seem too exorbitant to pay $1,000 for 20 years of life.

Chairman Wangensteen: Thank you, Dr. Morgan. I think it is important. If anybody has any useful ideas that can be implemented by this group, that is the very circumstance that brings us together. We come together not only to announce newfound information, but we come together for cooperative purposes. If any of you have ideas you are contemplating, this is a good place to announce them so that they can be screened by others during the 2 years or more that will elapse before the next meet-

ing. If the National Advisory Cancer Council thinks well enough of this, I suggest we have a fifth gastric conference at some time.

Dr. George Moore hit upon an unusual find in ascertaining an unexpected affinity of brain tumors for fluorescein. Dr. Brunschwig and others have examined other dyes. Dr. Hubbard from our clinic, together with Dr. Moore and others, have explored a large number of dyes without observing any suggestion of special affinity for cancers in various other organs. Dr. Moore stumbled rather fortuitously on fluorescein. Possibly somebody will come upon something else. I think we need a little hope, too.

Does anyone want to say something about the survey methods other than X-ray on achlorhydria?

Dr. Strong: There is some recent work done by Dr. Cliffton, who has developed, at Yale, a blood test in relation to the antiproteolytic properties of blood. It has not been perfected for publication up to the present time but should be considered. He claims that by a blood test he can tell the difference between gastric ulcer and gastric carcinoma. It is interesting to us in the experimental field that gastric lesions in mice of the Brs subline show the high value of human carcinoma.

Chairman Wangensteen: Thank you, Dr. Strong. In that connection, I would like to ask Dr. Richardson if he has enough confidence in his studies to advise patients in his clinic definitely to wait for a decision as to whether the patient may have gastric cancer or ulcer, on the basis of differentiation that he spoke of yesterday.

Dr. Richardson: What is that question again?

Chairman Wangensteen: I was just wondering how sure you could be on the basis of things you were telling yesterday concerning differential criteria between cancer and ulcer that you could advise the patient well.

Dr. Richardson: At the present time we will only state in our reports that there is ulceration within the gastric lumen, and we suggest continued study through further washings.

We feel that carcinoma is either present or absent. We do not say the findings are suspicious, because we feel that if we do, the surgeon will lose confidence in the test. On the basis of a suspicious finding, the surgeon may remove the stomach, and when he fails to find carcinoma, the pathologists will be a bit embarrassed. By being definite in our conclusions, we believe we may be able to convince the surgeons and the clinicians that we have something worthwhile. We are gaining ground. Dr. Moses E. Steinberg, a gastric surgeon, has his nurse wash out the stomach following each gastroscopy, and the contents are sent to us for study. Our findings are then returned to him. Eventually we receive his clinical or surgical diagnosis, and in this way we are able to determine our degree of correlation.

The studies that are in progress are in their infancy. We have instituted, for example, a screening process in our outpatient clinic. All individuals who are over 45 and who come in for stomach study are routinely given an alchool or a histamine type of gastric analysis. A stomach wash is taken at the conclusion of the multiple gastric aspirations, and the discussion today is based on the result of this cytohistological study.

Chairman Wangensteen: That is the kind of information we want. I hope you are right. I think if we were to cull the opinions of my conferees here, they would say to me that every gastric ulcer would be under suspicion.

There is one thing relating to the circumstance of susceptibility that I think bears on the question. Dr. Howes touched on it, namely, if you have a gastric ulcer, does it ever heal out with adult gastric epithelium? If it doesn't, and you leave that vulnerable, susceptible area there, why ever treat a patient medically for a gastric ulcer? I think it is a very good question, and when we have a little better factual answer for that question, if the ulcer heals with something other than gastric epithelium, a vulnerable place remains in the stomach, and one understands why the patient comes back with a gastric ulcer in the very same spot again.

Dr. Richardson: If I may add some more to that.

Chairman Wangensteen: Not at this time, please. We will see how much time we have. I would like to call on Dr. Davenport now.

Dr. Davenport: I am a complete layman in this subject, and perhaps you will forgive anything I say on the basis of my ignorance. I must say I am very disappointed in some of the aspects of this conference. There are two questions which arise in any scientific problem, what is going on, and what to do about it. I am sure that the second problem is in very safe hands. One can trust the pathologists, the surgeons, and the other clinicians to do the best thing about it possible. What is going on is not at all answered, and I am quite convinced that very few serious attempts are being made to find out what is really going on.

The whole history of science tells us that the applied aspect depends upon the fundamental. We use up our fundamental capital when we apply it, and I am certain that this problem, as in a great many others, is running out of our fundamental capital, if, indeed, we have not already exhausted it. I think that is a very sad state in which to exist, and I would like to plead for some more fundamental work. I don't quite know how we are to go about that, but I think it should be done.

We are all interested in what is going on in the parietal cells. There has been a great deal of talk about it here in the last 2 days. Three people in the world are working on what is going on in parietal cells. You will never find out what is happening in them by looking at eosin-stained sections.

I would like to see more people working on that subject; I would like to see more people besides Dr. Hollander, who is doing excellent work, studying what is happening to the mucin-formation in the stomach.

How are secretions being formed? How are the cells being resynthesized? This brings up the big problem of protein synthesis, the chemical change in mitosis. I think there are probably four or five people in the world working on that problem. I think we could spend fewer dollars on some aspects of this problem and a few more on the research fundamentals which underly it.

I would estimate, in my knowledge of another problem like this, that it would probably cost about a hundred thousand dollars for 10 years to find out if research is going to get any place. I don't think that is a great deal of money to be spent, and I would like, in this problem, as in all others, to see that the fundamental work is not neglected.

Chairman Wangensteen: At this juncture, I am going to call on Dr. Barrett. I presume he knows more about the world literature of gastric cancer than anybody else in the world. I suppose if one of us would set himself the task of accumulating the knowledge he has, he might die in the first alcoves. Yet the important thing is to bring out ideas which will be helpful to us. I am going to ask you specifically, Dr. Barrett, are there things that haven't gotten into this program that are important to this problem?

Dr. Barrett: Oh, yes, Dr. Wangensteen, there are some very important problems that haven't gotten into this conference! I often say that in cancer we are working in a field that is so dark that we don't even know how dark it is. I think one of the darkest fields in cancer is the theoretical aspect of gastric cancer. Dr. Wangensteen has very flatteringly attributed to me a knowledge of gastric cancer that I feel I hardly possess. I have tried to see all aspects and all opinions, but I am far more impressed with how much I found out that I didn't know than with how much I found out that I did.

I heartily agree with Dr. Davenport when he points out that all applied science depends upon fundamental observations. I am well aware of the fact that the man who sees gastric cancer from a clinical aspect must be so overwhelmed with the human problem involved that he is not apt to see through it to the fundamental aspects on which his work is based. Nevertheless, I think that a group such as this must look for fundamental attacks upon the problem as well as methods of handling the disease after it occurs in the patient.

One of the aspects of the gastric cancer problem that interests me very much is the possible relationship with anemia. It is a very difficult problem, and I don't know of any work being done on that aspect. Dr. Davenport complained that he knew of only three men working on the parietal cell, which I think is perhaps a slight under-

estimate although there certainly aren't many. I don't know anybody who is trying to explain to us how to tell the difference between the various anemias, particularly the macrocytic anemia that occurs in gastric cancer, from other types of macrocytic anemia, if there is any difference. There is some evidence for thinking that these are different. There is no proof that they are. It is a very difficult problem. I don't blame anybody for not wanting to take it up, because he is not going to find very many cases on which to work, and he is going to have a hard time getting an answer; but here is an aspect that is not being touched.

Dr. Morgan referred to one aspect that is not being studied very much. He suggests the use of a capsule containing barium, the capsule being so constructed that it will dissolve at selected pH's and release the barium, which I believe he means to be detected by X-ray. It is a splendid idea, as has been mentioned before. We don't need the X-ray if we can get the capsule, because, if we get the capsule, we don't need anything in it but a little methylene blue, or some such substance, to do our survey.

One of the aspects that is being touched upon only in a very limited number of places has to do with direct carcinogenesis in the stomach. We have become so familiar with the hydrocarbons in this field that we automatically mean, in most cases, hydrocarbons when we say carcinogenic chemicals. We are all aware of the fact that practically all of these are, shall we say, lipophilic substances. We are equally aware that among the tissues of the body which are exposed to the external environment, the stomach is noteworthy for being hydrophilic. I wonder if any of us seriously believe that human gastric carcinoma or spontaneous gastric carcinoma in any animal occurs by the exposure of such a highly aqueous tissue to such an insoluble substance, such as lipotropic substance as most of the chemical carcinogens. This suggests a whole field of chemical research to find if there is a group of carcinogenic substances that are more adaptable to this type of tissue. Perhaps our knowledge of the hydrocarbons, stimulated as it was by the English workers, studied as it has been in the skin, has led us astray here, and we have forgotten to look for an entirely different class of substances which might work quite well. There is some work being done on this aspect, and one of the most likely ideas that has been suggested, I believe Dr. Ivy suggested it about 2 years ago, would be to select some molecule, perhaps a dye, that we know is selectively secreted in the stomach and is hydrophilic and attach to it some molecule that might have carcinogenic influence.

The time is getting so short that I don't want to go on. There are other aspects. I think the greatest need, however, is not new fields to conquer in gastric cancer so much as it is workers who are willing to go into the field. When we get more and more workers who are competent and willing to attack the fundamental problems of gastric cancer, we will have ample time to look for some more fields in which to put them.

Chairman Wangensteen: Thank you, Dr. Barrett. At this juncture I am going to ask if any members of the Gastric Cancer Conference would like to say something additional? Would you, Dr. Comfort?

Dr. Comfort: No, except that I would like to join Dr. Davenport and Dr. Barrett in emphasizing the paucity of fundamental knowledge that is coming to the physician and surgeon in helping to solve the problem. I hope this group will put the emphasis where I believe it is needed.

Chairman Wangensteen: I see Dr. Stone is still here. I think that we of the conference owe a vote of thanks to representatives of both the University of California and Leland Stanford Medical School for the hospitality they have extended and the opportunity of meeting here.

Is Dr. Meader here? Dr. Meader and members of the National Advisory Council, when a meeting like this, designed to stimulate interest, comes to a conclusion, I fear that we have not presented you with handles by which the problem can presently be shaken down effectually. But we shall continue the search for such handles.

If you will second my suggestion, I think we should give some applause to members of the California group in both institutions. (Applause.)

I see Dr. Heller is here. He began the conference, and I will ask him to conclude it.

Dr. Heller: Thank you. Dr. Wangensteen, ladies and gentlemen, I certainly think that time and the record will establish the value of a conference such as this. Certainly it bespeaks the desire on the part of the scientists generally in this difficult field to stimulate action.

I am hopeful that soon we can translate this into practical years added to life. Certainly, on behalf of the National Cancer Institute, I should like to thank Dr. Wangensteen and Dr. Barrett and members of the committee for their work and the California hosts for their very generous hospitality and for the opportunity of getting together. Thank you very much.

(Whereupon, at 1:10 o'clock p. m., December 14, 1948, the Fourth Conference on Gastric Cancer adjourned.)

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